SERUM, URINE AND ELECTROCARDIOGRAPHIC CHANGES IN THE DIAGNOSIS AND DIFFERENTIATION OF Q-WAVE AND NON Q-WAVE MYOCARDIAL INFARCTION

PAUL ANTONY KELLY

DOCTOR OF MEDICINE

THE UNIVERSITY OF EDINBURGH

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DECLARATION

I declare that this thesis has been composed by myself, and that the work contained therein is my own.

Signed

Paul Antony Kelly
May, 1995

ABSTRACT

Thrombolytic therapy (TT) significantly reduces mortality and morbidity following acute myocardial infarction (AMI). This is presumed to be secondary to reperfusion of the infarct related artery. Increased use of TT has produced a requirement for early, accurate diagnosis of AMI. Not all patients show equivalent benefit to TT; consequently, the management of patients post-AMI is likely to become increasingly varied. There is a need for non-invasive assessment of arterial patency to identify patients in whom myocardium remains at risk.

To compare 4 recognised biochemical markers, creatine kinase-MB (CK-MB), mass and activity, myoglobin and troponin-T to serum and urinary creatine concentrations in the diagnosis and differentiation of AMI, and the non-invasive assessment of arterial patency following thrombolysis, 191 patients admitted to a coronary care unit with a differential diagnosis of AMI were studied. On the basis of the admission electrocardiogram (ECG), they were divided into the following groups:

Group A: Admission ECG diagnostic of AMI, (n=56)

Group B: Admission ECG non-diagnostic of AMI but AMI proven at day 3, (n=48)

Group C: Admission ECG non-diagnostic of AMI, AMI excluded at day 3, (n=87)

Type 1: Q-waves on day 3 ECG (n=58).

Type 2: No Q-waves on day 3 ECG (n=46).

Patients in group A were considered to have achieved successful reperfusion if they had $\geq 50\%$ resolution of ST segment elevation within 2 hours of TT.

RESULTS

Serum and urine creatine concentrations were not discriminatory in the diagnosis of AMI. The other 4 markers were able to differentiate all subgroups from non-AMI with a high degree of sensitivity and specificity (range 80-98% and 94-99% respectively); troponin-T having the greatest diagnostic power for all AMI (groups A and B) and group A, $p<0.05$.

CK-MB mass was best at detecting group B and type 2 infarction, $p<0.05$.

Myoglobin was able to identify AMI faster than CK-MB activity in all patient groups, $p<0.01$ and detect all infarcts, group A and type 1 infarction faster than troponin-T, $p<0.04$. CK-MB mass was more rapid than CK-MB activity for all AMI diagnosis, $p<0.05$.

Patients in group A who reperfused were diagnosed most accurately by myoglobin at 2 hours after TT, $p<0.05$, and by CK-MB mass at 4 hours after TT, $p<0.05$.

Myoglobin concentrations peaked significantly earlier than all other markers in reperfusers, $p<0.001$. All 4 markers showed significantly earlier peaks in reperfusers than non-reperfusers, $p<0.001$.

CONCLUSIONS

Serum or urine creatine concentrations are not useful diagnostic markers for AMI

CK-MB mass and activity, myoglobin and troponin-T are all highly sensitive and specific in AMI diagnosis, troponin-T having an advantage over CK-MB activity and myoglobin.

Myoglobin provides the earliest diagnosis of AMI, and the most rapid biochemical indication of reperfusion following thrombolytic therapy.

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1.1 INTRODUCTION

Acute myocardial infarction (AMI) is a common cause of mortality and morbidity (Kannel,1990). In England and Wales, 89,336 deaths were reported in 1990 (HMSO,1990).

In the past 10-20 years there has been a considerable amount of research into this subject. As the pathophysiology of acute infarction has become more clearly understood, so the associated clinical, electrocardiographic and enzymological features of AMI have been re-evaluated.

In essence, AMI is part of a heterogeneous spectrum of a presumed common pathology, namely acute occlusion of a coronary artery. This manifests itself as the acute coronary syndromes, that is, unstable angina through AMI to sudden cardiac death (Willerson,1986).

The diagnosis of AMI is traditionally made from the triad of chest pain, electrocardiogram (ECG) changes and a rise in certain, so-called, cardiac enzymes. The presence of any two of these criteria being regarded as sufficient to confirm the diagnosis (World Health Organisation criteria (WHO,1962)).

There are a number of problems inherent within this approach. Firstly, the pain associated with AMI is not always typical and in some instances may be absent, so-called silent infarction (Kannel,1984). Secondly, ECG changes diagnostic of AMI may not be present; in up to 60% of cases in some series (Lee,1989). Thirdly, the rise in serum concentration of certain enzymes has an in-built temporal delay which implies that for some markers at least 10 to 16 hours must pass from the time of onset of AMI before this rise can be detected (Hutler,1981; Sharkey,1988).

Recent large scale clinical trials of thrombolytic therapy administered soon

after the onset of AMI have shown significant reductions in both mortality and morbidity (GISSI,1987; ASSET,1988; ISIS-2,1988; AIMS,1988). These trials also showed that the earlier that thrombolytic therapy was given, the greater the benefit.

As new technologies such as percutaneous transluminal coronary angioplasty (PTCA) have been developed, and the number and use of pharmacological agents expanded, the potential to intervene positively in the process of AMI has become apparent (O'Neill,1987; Meyer,1982; Hartzler,1983). Consequently the management and prognosis of AMI has changed significantly.

Any information pertaining to the patency of the infarct related artery is clearly very useful when such a potential to intervene exists. Primarily, the diagnosis of AMI is required, but in addition to this, an indication as to whether reperfusion or re-occlusion of the artery has taken place will further influence management.

These matters will be considered in greater detail below.

1.2 PATHOLOGY OF ACUTE MYOCARDIAL INFARCTION

The atherosclerotic lesion common to all acute coronary syndromes is the raised fibro-lipid plaque present in large and medium-sized arteries (Singh,1984). The nature of the plaque can vary considerably from being fibrous-rich (consisting mostly of collagen and smooth muscle cells), to being lipid-rich (consisting mostly of lipid-filled foam cells). A spectrum of plaque types between these two extremes exists (Hangartner,1986; Ambrose,1988).

The presence of a plaque within the vessel wall results in a reduction in luminal diameter. A slow progression in reduction of diameter is thought to be responsible for the symptoms of chronic stable angina which occur at a relatively fixed degree of exertion for any one patient. When a plaque has caused a reduction in lumen diameter of at least 70%, it is termed a high grade stenosis (Willerson,1984). If the plaque occupies the complete circumference of the vessel wall it is termed a concentric lesion; if it occupies only part it is described as eccentric (Hangartner,1986; Ambrose,1985). Since part of the intima in eccentric lesions is unaffected by the plaque it is subject to vasospastic influences and the potential for an acute alteration in luminal diameter exists. The morphology of individual plaques is also seen to vary, thus, a type I stenosis is smooth in outline, whereas a type II stenosis has an uneven contour. As will become apparent, these qualitative assessments of coronary artery anatomy do appear to be related to events taking place at the time of infarction.

Coronary angiography following AMI demonstrates that thrombus formation resulting in total (no forward flow) or sub-total (95% obstruction) vessel occlusion is the principal cause of AMI (DeWood,1980). The majority of occlusions are

secondary to a deep focal endothelial injury, namely ulceration or rupture of the plaque with resultant intimal haemorrhage and exposure of collagen and lipid (Ridolfi,1977; Davies,1985; Fuster,1988; Davies,1989; Onodera,1989). This provides a profound thrombogenic stimulus (Fuster,1988), and thrombus formation results in subsequent expansion of the plaque (Davies,1985). The actual mechanism of initiation of thrombus formation is not entirely understood, but the majority of thrombi occur at predominately lipid-filled plaques (Hangartner,1986), and are more likely to form at a type II rather than a type I lesion (Roberts,1972; Ambrose,1985).

Although serial angiographic studies (pre- and post-AMI) up to 7 years apart have shown that arterial occlusion does not always take place at previously identified high grade stenoses (Singh,1984; Ambrose,1988; Haft,1988), the risk of vessel occlusion is related:

a) to the complexity of the underlying lesion after rupture, that is, if it occupies an irregular profile within the lumen and therefore a large surface area on which thrombus may form, and b) to the presence of local spasm (as may occur in eccentrically sited plaques) (Oliva,1977; Maseri,1978,1986; Gibson,1986a).

The effect of vessel spasm is increasingly recognised as having a significant role in the pathogenesis of acute coronary syndromes (Willerson,1986; Maseri,1978). Endothelial injury results in loss of production of naturally occurring vasodilators such as prostaglandin I_2 (PGI_2). The local release of thromboxane A_2 (TXA_2) and serotonin by platelets further promotes vasoconstriction and platelet aggregation (Willerson,1984; Haft,1988).

There is often a stuttering process to the development of complete coronary artery occlusion which may be manifest clinically, usually by variation in severity

of pain (Braunwald,1988). This often places in doubt the exact moment of infarction. This has important clinical consequences because of recent developments in the management of AMI. This will be described more fully below.

In a comparison of patients presenting less than (early), and greater than (late), 6 hours from the onset of chest pain, Beek found a considerable overlap with regard to ECG and enzyme characteristics (Beek,1991). He concluded that some patients supposedly presenting "late" after infarction in fact have evidence of recent arterial obstruction and so would benefit from reperfusion therapy. This is further verified by the observation that the development of histological changes of infarction seen in post-mortem studies occur over a greater number of hours than those seen in animal studies when ligation of a coronary artery has been used as the model of infarction (Wassermann,1989; Maseri,1986). The development of the pathological changes of AMI appears to be in 2 phases, an evolving phase and a convalescent phase (Pepine,1989). Restoration of blood flow to the myocardium during the evolving phase may prevent, either partially or completely, some of the changes of infarction.

The success of myocardial salvage is dependent upon a number of factors including the presence of a good collateral circulation (Fujita,1991), low heart rate and low blood pressure (rate product). It follows that if the process of coronary artery occlusion is intermittent, and a number of variables further affect the time taken for myocardium to become non-viable, then the time window for success of thrombolytic therapy is potentially greater than that predicted from animal studies (Wassermann,1989).

It is also now recognised that the effects of infarction are not simply necrosis of the tissue supplied by the affected vessel. Thinning of the infarct zone and its subsequent enlargement (infarct expansion) result in changes in structure and function in myocardium distant from the site of infarction (Golia,1994). This process is termed ventricular remodelling and is first seen to occur during the convalescent phase. It has been proposed that although late administration of thrombolytic therapy will not be able to restore blood flow to the infarcted area to prevent those changes seen in the evolving phase, it may have beneficial effects with respect to preventing infarct expansion and the subsequent complications associated with extensive ventricular remodelling; namely impaired left ventricular function and arrhythmogenesis (Podrid,1992; Golia,1994).

In summary, the intermittent nature of vessel occlusion and the benefit of restoring myocardial flow provide strong reasons for being able to rapidly and accurately diagnose AMI, and the degree of patency of the infarct related artery; the latter preferably by non-invasive methods.

1.2.1 Q-WAVE AND NON Q-WAVE MYOCARDIAL INFARCTION

Acute myocardial infarction has traditionally been categorised as transmural or subendocardial by pathologists (Marmor,1982). This pathological classification does not bear close correlation to ECG changes and currently the clinical classification of patients with AMI into Q-wave AMI (QAMI) and non Q-wave AMI (NQAMI) is preferred (Boden,1989a,b; Spodick,1988); that is, whether AMI results in the development of Q-waves on the 12-lead ECG.

One distinct advantage of this change in nomenclature is that by using a

classification which is readily apparent from a non-invasive test, namely, the electrocardiograph, clinical trials and patient management can be developed with a significant degree of conformity between investigators. The difficulty of using a pathological classification is that the true diagnosis can only be made at post-mortem studies with the accuracy and/or validity of data verified in only a small proportion of patients. Phibbs argues that this differentiation of AMI into QAMI and NQAMI has no pathological basis and urges that it be refrained from (Phibbs,1983). However, he makes no suggestion as to what, if anything, should be used in its place. As will become apparent, considerable differences between these two groups of patients do exist. Since the majority of workers currently accept this differentiation of patients, this classification will be used for the remainder of the discussion.

1.2.1.1 PATHOPHYSIOLOGICAL DIFFERENCES BETWEEN Q-WAVE AND NON Q-WAVE MYOCARDIAL INFARCTION

QAMI is thought to occur when there is a complete and persistent obstruction to flow and the whole of the myocardium in any given zone is seen to infarct. New Q-waves are then identified on the ECG, typically during the first 3 days after the onset of symptoms (Coll,1988).

NQAMI results when some degree of myocardial perfusion is maintained by either anterograde (Davies,1985; Goldberg,1987) or collateral flow (Fuster,1979; Ferlinz,1990; Dwyer,1990). Anterograde flow may be maintained by a reduction in the degree of vasospasm or by spontaneous recanalisation resulting in early reflow (Boden,1991). Angiographic studies have shown that the restoration of flow must be complete (Thrombolysis in Myocardial Infarction (TIMI) grade 3) as

opposed to partial (TIMI grade 2) in order to detect any variation in infarct size or ECG classification with respect to QAMI (Karagounis,1992). NQAMI constitute 20-40% of all AMI (Abbott,1973; Gibson,1986a; Goldberg,1987; Huey,1987).

Although some degree of vessel perfusion persists in NQAMI this is not sufficient to completely prevent myocardial necrosis. Experimentally complete obstruction to flow must be of less than 2 hours duration for NQAMI to result and greater than 2 hours for QAMI to result (Ferlinz,1990). Thus, NQAMI has been described as an incomplete or aborted QAMI (Boden,1989b,1991) resulting in some degree of salvage of epicardial myocardium. It appears also to be a predictor of greater clinical instability due to the presence of viable but threatened myocardium within the perfusion zone of the infarct related artery (Gibson,1986b). This is supported by the observation that coronary angiography at 1 and 7 days after NQAMI showed that the number of vessels occluded actually increased, whereas angiographically at similar intervals following QAMI a decrease in the number of vessels occluded was noted (DeWood,1986; Marmor,1982). Peak creatine kinase (Krone,1983) and total CK release (Stone,1988) are lower in the NQAMI group, indicating smaller infarct size (Chouhan,1991).

Interestingly, coronary angiography reveals no significant differences in coronary artery anatomy between QAMI and NQAMI (Krone,1983; Schulze,1978). This supports the evidence outlined above that a number of characteristics of the plaque other than size have a significant influence on the degree of obstruction of the lumen during the acute event, and hence the extent and duration of vessel occlusion (Schweitzer,1990a).

1.2.1.2 CLINICAL DIFFERENCES BETWEEN Q-WAVE AND NON Q-WAVE MYOCARDIAL INFARCTION

Although the classification of QAMI and NQAMI is based upon a simple non-invasive test, namely the 12-lead ECG, a large number of clinical differences between these 2 groups of patients have been identified, supporting the observations that pathological differences exist and raising the possibility of differing therapeutic approaches in their management (Boden,1989b).

The first AMI experienced by any given patient is unlikely to be a NQAMI (Benhorin,1990). Prodromal symptoms such as sweating, nausea and vomiting are more likely with NQAMI (Ingram,1980; Scheldt,1976). In-hospital mortality is significantly lower for NQAMI versus QAMI (4-12% v 9-25%) (Marmor,1982; Goldberg,1987; Stone,1988). Most studies show that late mortality increases dramatically in the NQAMI group to 12% by year 3, and continues at 12% for each subsequent year (Krone,1983). Although Goldberg showed no significant difference between NQAMI and QAMI patients he did confirm a trend to increased late mortality in the NQAMI group (Goldberg,1987).

Late cardiac events are more common in the NQAMI group. For example, reinfarction has been reported to be as high as 57% by year 5 in the NQAMI group, compared to 15% in the QAMI group (Spodick,1983; Stone,1988). Twice as many patients with NQAMI require PTCA or coronary artery bypass grafting surgery (CABG) than those with QAMI (Gibson,1986b).

In-hospital morbidity also differs significantly between the 2 groups. Infarct expansion is more frequently observed in the QAMI group. Congestive cardiac failure (CCF) and serious atrial and ventricular arrhythmias are observed more

frequently in the QAMI group due to the larger amount of myocardium infarcted during the acute event (Taylor,1980). This is also manifest by increased wall motion abnormalities in patients with QAMI (Hutter,1981; Horowitz,1982), and overall impaired left ventricular function (Chouhan,1991).

Within each of the 2 groups, patient subsets are identified. Thus, patients with anterior QAMI have an increased incidence of CCF compared to those with inferior QAMI (Benhorin,1990). Those patients with a peak aspartate aminotransferase (AST) level $\leq$240 Units/Litre (U/L) had an in-hospital mortality of 3%, whereas patients with peak AST $>$240 U/L had a much higher in-hospital mortality of 11% (Krone,1983).

Within the NQAMI group, patients less than 60 years of age have an excellent prognosis, whereas patients greater than 60 years of age have a 12% mortality by 3 years, and an 11% yearly mortality thereafter (Goldberg,1989). Patients with ECG changes affecting 2 or more leads, or with a magnitude of at least 2mm ST segment shift have a greater incidence of recurrent ischaemia, infarction and death compared to those with more subtle ECG abnormalities (Cohen,1991).

Patients identified as having sustained a NQAMI at day 3 with ST segment depression on their admission ECG have a significantly worse prognosis than patients presenting with ST segment elevation or no significant ECG changes (Willich,1987; Dewhurst,1991; Boden,1991; Abbott,1973). Ogawa demonstrated that those with ST segment depression have a higher incidence of three vessel disease, cardiogenic shock and death (Ogawa,1985). Patients with ST segment depression compared to ST segment elevation were older, had an increased

incidence of diabetes mellitus, hypertension, previous MI or angina and congestive cardiac failure (Willich,1987).

Overall, there seems little doubt that patients with QAMI and NQAMI do differ significantly, and also that within each of these categories of AMI different subsets exist. The development or lack of development of Q-waves following AMI has a marked effect on the predicted in-hospital and post-discharge mortality and morbidity. As the management of patients following AMI is increasingly likely to exploit these differences, the ability to classify these patients early in their clinical course may be of clinical benefit.

1.2.2 THROMBOLYTIC THERAPY AND ACUTE MYOCARDIAL INFARCTION

Only relatively recently has it become accepted generally that opening coronary arteries at the time of AMI could benefit prognosis (Rentrop,1979). Several large multi-centre, multi-national, double-blind, placebo-controlled trials of intravenous thrombolytic therapy in acute myocardial infarction have demonstrated significant reductions in mortality and morbidity if given within hours from the onset of chest pain (GISSI,1987; ASSET,1988; ISIS-2,1988; AIMS,1988). Comparison of the three currently available preparations, streptokinase (SK), tissue plasminogen activator (tPA) and anisoylated plasminogen streptokinase activator complex (APSAC) showed no overall advantage with respect to 30 day mortality for any individual agent (ISIS-3,1992), similarly there was no advantage demonstrated between tPA and SK at 6 months (GISSI-2,1990). These findings have been questioned more recently by the Global Utilisation of Streptokinase and Tissue

Plasminogen Activator for Occluded Coronary Arteries (GUSTO) study, in which a reduction in 30 day mortality was seen for an accelerated tPA with intravenous heparin regime, compared to SK with either subcutaneous or intravenous heparin (GUSTO-I,1993). However, all patients in GUSTO had ST segment elevation on the admission ECG, all received thrombolytic therapy within 6 hours of symptom onset, and perhaps most importantly, the trial was not double-blind in design, unlike ISIS-3 and GISSI-2. These differences in study design may explain this disparity in observed mortality reduction.

What is apparent from all trials of thrombolytic therapy in AMI, is that benefit is greatest the earlier that thrombolytic therapy is administered after the onset of symptoms (O'Rourke,1988). Recent studies of out of hospital thrombolysis, or even percutaneous transluminal coronary angioplasty suggest that the first 100 minutes following the onset of symptoms are of paramount importance to initiate therapy aimed at restoring arterial patency (Weaver,1990; GREAT,1992; Ziljstra,1993).

The side effects of thrombolytic therapy are relatively low in number but can be serious, especially if given in clinical situations that can mimic AMI, for example, dissection of the aorta (Satler,1984; Blankenship,1989) and bacterial endocarditis (Herzog,1991). The need for not only rapid, but also accurate diagnosis of AMI, is of marked clinical importance.

1.2.2.1 CORONARY ARTERY PATENCY FOLLOWING THROMBOLYSIS

All thrombolytic agents increase the conversion of plasminogen to plasmin, although tPA and APSAC are more fibrin-specific than SK (Verstraete,1987).

Intravenous thrombolytic therapy reverses some of the processes of thrombus formation. In general, thrombolysis is more successful when plaque disruption is superficial (Onodera,1989). This is explained by the observation that dissolution of the fibrin network and fibrin-rich material (which lies distally) is relatively easy, whereas removal of the intra-plaque platelet-rich component is more difficult (Hugenholtz,1993).

The immediate end-point of successful administration of thrombolytic therapy is patency of the infarct related artery. An important series of studies investigating this area is the Thrombolysis in Myocardial Infarction (TIMI) studies. They defined the degree of reperfusion into 4 grades, (0, absent anterograde flow; 1, penetration of the thrombus by contrast material but incomplete filling of the distal vessel; 2, complete opacification of the distal vessel with delayed filling or washout; and 3, normal vessel flow) (TIMI,1985).

Patency rates differ between different thrombolytic agents. The GUSTO-1 trial demonstrated that the greatest vessel patency at 90 minutes after initiation of thrombolytic therapy was seen for an accelerated tPA regime; 54% had TIMI grade 3 flow and 27% TIMI grade 2 flow. Whereas following SK, 30% had TIMI grade 3, and 25% TIMI grade 2 flow (GUSTO-1,1993). Accelerated tPA resulted in 13% more patients with TIMI grade 3 flow than if tPA was given at a fixed rate (Simes,1995). Earlier studies had shown patency rates to be of the order of 60-80% if thrombolytic therapy was given within 4-6 hours of the onset of chest pain (Davies,1984; TIMI,1985; Maseri,1986; O'Neill,1987), with overall early coronary patency following tPA exceeding that of SK by a ratio of 1.4 (Granger,1992). It has also been demonstrated consistently that patency is greater following intra-coronary

(72-96%) (Weinstein,1982), compared to intra-venous (45-65%) (Spann,1982) injection. Interestingly, patency is higher when treating occlusions of the left anterior descending artery (LAD) compared to the right coronary artery (RCA) (TIMI,1985; Schroder,1987; Bates,1988).

Coronary angiography following AMI has revealed that vessel patency increases with time even if a thrombolytic agent is not administered, although the proportion of vessels opened is much lower than that seen if thrombolysis is given. It has been proposed that this phenomenon is due to secondary spontaneous thrombolytic activity or to a reduction in the degree of vasospasm (Pichard,1981; DeWood,1986; Gibson,1986a). This may be the difference between QAMI and NQAMI, that is, all infarcts result initially from total vessel occlusion but the degree of occlusion can then vary, with subsequent variation in pathological outcome.

Huey et al showed 23% of NQAMI related arteries to be patent at the time of angiography (Huey,1987). He argued that when patency rates for NQAMI approach those observed in patients studied after thrombolytic therapy, the need for thrombolysis in these patients can quite rightly be questioned. This has some validity, but clearly will not be true for all NQAMI and therefore some patients are still likely to benefit from thrombolysis. Considering the incidence of AMI, and from above, that at least 30% of patients placed into this supposedly favourable prognostic group on admission will develop features of Q-wave AMI by day 3 (and therefore be placed into a group with a considerably worse early prognosis), the converse argument that all patients with myocardium at risk of infarction should be protected by the administration of thrombolytic therapy where this has been shown to be of benefit can be proposed. This standpoint is currently being investigated by

the GUSTO-II trial evaluating the comparison of intravenous heparin and recombinant hirudin for acute coronary syndromes. Although the doses of these agents have been modified during the trial (GUSTO-IIa,1994), the investigators are assessing the relative effect of these 2 treatments upon unstable angina, NQAMI and ST segment elevation AMI, the concept of "thrombolytic therapy" being applied to all pharmacological manipulations of the thrombotic process, not only the 3 recognised agents of SK, APSAC and tPA.

1.2.2.2 WHICH PATIENTS BENEFIT FROM THROMBOLYSIS?

Infarct size is the main determinant of survival following AMI (Braunwald,1987; Serruys,1987); this would be expected to lend weight to the concept that any degree of myocardial salvage is of benefit. However, this has not been confirmed as being of statistical benefit by clinical trials. The ISIS-2 study of SK and/or aspirin versus placebo in AMI in which ECG changes were not required for the administration of thrombolytic therapy to take place, (clinical suspicion of AMI being the main entry criterion), showed by retrospective sub-group analysis that patients with QAMI benefit significantly more from thrombolytic therapy than those with NQAMI, the latter having no improvement in survival when compared to the placebo group (ISIS-2,1988). However, patients identified at day 3 as having NQAMI who had no changes on the admission ECG had a 50% reduction in mortality with SK compared to placebo (ISIS-2,1988; Boden,1989c). Those patients with an abnormal ECG other than ST elevation or depression had a 38% reduction in mortality compared to placebo, including patients with prominent R waves in V_1 and V_2, where this was thought to represent a posterior AMI (Anderson,1993).

Therefore, it would appear that certain patients without "classical" ECG changes of ST segment elevation on the admission ECG do benefit from thrombolytic therapy and consequently it can be argued that there is clinical worth in identifying patients within this group, who otherwise would not be identified sufficiently early as having sustained AMI, and would subsequently be treated suboptimally.

The degree of reperfusion obtained appears to significantly affect prognosis. Thus, patients in whom TIMI grade 3 flow is seen at angiography following the administration of thrombolytic therapy have a better prognosis than patients in whom TIMI grades 1 and 2 is seen (Badger,1987). The GUSTO angiographic substudy has shown a significant relation between 30 day mortality and vessel patency at 90 minutes. Thus, mortality at 30 days for TIMI grades 0 and 1 (no patency), was 8.9%, for TIMI grade 2, 7.4% and TIMI grade 3, 4.4% (GUSTO-I,1993a). As well as showing that differences in vessel patency influence 30 day mortality, this study also revealed differences in early mortality depending upon the degree of reperfusion. Mortality at 24 hours was highest in those patients with TIMI grade 2 flow at 90 minutes, 2.93%, compared to 2.35% for patients with TIMI grades 0 and 1, and 0.89% for patients with TIMI grade 3 flow (Simes,1995). Why patients with TIMI grade 2 flow have the worst immediate prognosis is not clear. It has been proposed that this flow pattern represents microvascular complications distally, possibly secondary to myocardial necrosis and localised oedema. This degree of reperfusion may therefore act as a marker, rather than a cause, of less favourable outcome (GUSTO-I,1993a; Hugenholtz,1993).

These findings have led to the concept of the "open artery hypothesis", namely that the success of early reperfusion is the primary determinant of difference

in outcome between the treatment groups. (Topol,1993; Kennedy,1995). This is further supported by the fact that it was the degree of reperfusion that determined prognosis, not the specific thrombolytic regime achieving that particular effect (Simes,1995). The general observation that up to 30% of infarct related arteries will not be reperfused with intravenous thrombolytic therapy, and the suspicion that the degree of vessel patency was of marked prognostic significance has led to the evaluation of primary PTCA in the management of AMI. The safety of this technique was demonstrated in the Primary Angioplasty Registry (Brodie,1994), and in a large study of 1,000 consecutive patients (O'Keefe,1993). The Primary Angioplasty in Myocardial Infarction (PAMI) trial compared PTCA with tPA in 395 patients within 12 hours of the onset of symptoms (Stone,1995). This study showed that in-hospital, and 6 month, death and re-infarction rates were lower for primary PTCA compared to tPA, 5.1% vs 12%, and 8.2 vs 17% respectively. TIMI grade 3 flow was restored in 95% of patients in the PTCA arm of this study. Zijlstra et al showed that late patency (3 months) was higher in a PTCA treated group, and proposed that this accounted for the improved clinical course in these patients compared to those receiving SK (Zijlstra,1993). Similar results were shown by Brodie et al with 87% of patients having TIMI grade 2 or 3 flow at 6 months (Brodie,1994). The findings of PAMI were not supported by Rogers et al who found no benefit for PTCA compared to thrombolytic therapy (Rogers,1994). Similarly, the addition of PTCA to thrombolytic therapy does not appear to confer clinical benefit (Michels,1995). Although primary PTCA would seem to be the absolute means of restoring vessel patency, the availability of this technique is likely to be limited to a relatively small number of centres with the facilities and expertise to

deliver such a service. What these studies do provide is an indication of the potential reductions in mortality that may accrue if pharmacological methods to restore patency increase in efficacy.

Another indication of the impaired survival resulting from failure to restore vessel patency was seen in a study by Cheriex et al which showed that myocardial rupture typically occured in patients in whom reperfusion did not take place (Cheriex,1995).

Although early administration of thrombolytic therapy is undoubtedly favourable (in theory completely reversing the changes of the evolving phase outlined above), especially if administered within 100 minutes of symptom onset, later administration of thrombolysis does appear to confer some clinical benefit. The precise mechanism of this is unclear, but it has been reported to limit overall infarct size (Richardson,1989), as well as influence the process of ventricular remodelling, so having a beneficial effect on LV function (O'Rourke,1988; Ferlinz,1990). It has also been suggested that this will reduce the arrhythmogenic potential of the infarct related area, and so improve prognosis (Ambrose,1993).

1.2.2.3 RE-OCCLUSION OF THE INFARCT RELATED ARTERY FOLLOWING THROMBOLYSIS

The patency of the infarct related vessel following AMI is variable. Coronary artery re-occlusion is seen in up to 33% of patients receiving thrombolytic therapy (Lim,1991), and is especially common where high grade stenoses are identified (Davies,1985), or where flow following thrombolytic therapy is low (TIMI grades 1 and 2) (Badger,1987; Hellstrom,1991). Re-occlusion is more common in inferior (20%) than anterior (9%) AMI (Hugenholtz,1987; Schroder,1987; Bates,1988). The

fact that up to a third of successfully opened vessels re-thrombose suggests that a thrombogenic stimulus persists after administration of thrombolytic agents in a significant number of patients. Findings from the angiographic substudy of GUSTO-I showed that early (90 minute) assessment of flow in the infarct related artery could not predict which vessels would re-occlude by day 7, nor did it appear to be related to the angiographic appearance of the intra-coronary lesion (Reiner,1994). However, it needs to be recognised that this study took place only in patients with ST-segment elevation, who presented early (within 6 hours of symptom onset). Also, the mean time to treatment in GUSTO-I was 2.7 hours after the onset of symptoms. These differences from earlier studies may account for the variations in angiographic data, however, it does not hide the fact that a significant number of patients will re-occlude after achieving successful reperfusion, and so lose the benefit of restoration of arterial patency.

The observation from ISIS-2 (ISIS-2,1988) that the addition of aspirin to streptokinase was synergistic (using early mortality as an endpoint) suggests that inhibition of platelet function is important in the period immmediately post-infarction (Davies,1985), and more aggressive thrombolytic regimes are currently under investigation.

1.2.2.4 CLINICAL IMPLICATIONS OF CORONARY ARTERY RE-OCCLUSION

Infarct extension is defined as the occurrence of a new event, namely, chest pain, arrhythmia or haemodynamic impairment when accompanied by new ECG changes and or further elevation of cardiac enzymes (Fraker,1979). A prerequisite for this to occur is repeat arterial obstruction with insufficient development of

collateral flow to prevent increased tissue infarction occurring. It is seen in up to 18% of patients post AMI, and usually occurs within 3-10 days after the first AMI (Fraker,1979; TIMI,1985; Marmor,1982). Within this time frame, several studies have shown it to be up to 3 times more common in NQAMI than QAMI (Marmor,1981; Gibson,1988; Richardson,1989; Benhorin,1990). Other risk factors have been identified, namely female gender, obesity and recurring chest pain (Marmor,1981). Patients sustaining infarct extension have a greater in-hospital (36% v 9%), and 1 year mortality (24% v 9%) than equivalent patients with an uncomplicated recovery (Marmor,1982).

Re-occlusion has important prognostic consequences since patients in this sub-group lose all the benefit of thrombolytic therapy (Lim,1991), having significantly impaired LV function compared to patients with patent infarct related arteries and an increased incidence of late potentials (Vatterott,1991). It follows that maintaining patency is important and by implication, detection of re-occlusion is of great clinical relevance.

The management of patients with coronary artery re-occlusion or infarct extension is difficult and currently controversial. Generally it is agreed that thrombolytic therapy is the best approach to limiting a further increase in infarct size and should be instituted as early as possible. However this can be far from straightforward, especially if thrombolytic therapy has been given previously. The role of rescue or salvage PTCA in these patients has been studied and there appears to be a consensus that if there is good evidence that reperfusion has not occurred, especially in patients with a first anterior MI (Ellis,1994), or in patients with cardiogenic shock, large infarctions, contraindications to thrombolytic therapy or

previous bypass graft surgery (O'Keefe,1993) that this is a valid technique with significant improvement in clinical outcome.

It can now be appreciated that accurate diagnosis of each acute event as it presents is of paramount importance, for example, failure to reperfuse adequately, and that, preferentially, this assessment will be performed non-invasively. With an increasing array of techniques and interventions available immediately post-AMI, this will allow appropriate and speedy management to be implemented.

1.2.3 SUMMARY

As the pathogenesis of acute coronary artery occlusion has become more clearly understood, it seems that certain events are common to all acute coronary syndromes. However, the outcome of such events varies considerably and whether infarction occurs, either transmural or subendocardial (as a pathological diagnosis), is dependent upon a number of factors. The process of infarction is not an all or none phenomenon; it is dynamic, and the outcome of thrombolysis, even if initially successful, is not guaranteed to remain so. The concept of the "open artery hypothesis" has led to the desired therapeutic aim of achieving complete reperfusion in all patients with AMI, new methods for realising this are currently being assessed. It can be hoped that advances in the development of thrombolytic agents, adjunctive therapies and the role of PTCA will lead to further reductions in mortality to add to the considerable achievements that have been realised in the past decade or so.

Methods available for the diagnosis of infarction and the non-invasive identification of vessel reperfusion and/or re-occlusion will now be considered.

1.3 DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

1.3.1 INTRODUCTION

Currently, the diagnosis of AMI is based upon a triad of chest pain, recognised changes in the resting 12 lead ECG and a rise in the serum concentration of certain biochemical markers. The presence of any 2 of this triad by the third day after presentation is regarded as sufficient to confirm the diagnosis of AMI (WHO,1962). With the advent of widespread use of thrombolytic therapy, the requirement for rapid diagnosis of AMI has developed, as well as the need for detection of post-thrombolytic events such as reperfusion and re-occlusion of the infarct related artery.

Each of the three components of the diagnostic triad will now be considered.

1.3.2 CHEST PAIN

Patients presenting to the Accident and Emergency department with chest pain constitute a large component of all acute medical cases, in one series up to 19% (Karlson,1991). The task of the admitting physician when making the diagnosis of AMI is firstly to discriminate pain that is compatible with a cardiac aetiology, and then to further subdivide this group of patients into those with AMI and those without. The consequences of mis-diagnosis in this group of patients can be disastrous, with significant medico-legal implications (Zarling,1983; Rusnak,1989); consequently the percentage of patients admitted to a high dependency area such as the Coronary Care Unit (CCU) who are later found not to have sustained an AMI can be as high as 70% (Gaspoz,1991).

Since chest pain and a 12-lead ECG are the 2 most readily available

diagnostic aids in the acute setting, a number of investigators have attempted to formulate protocols for stratifying patients at the time of presentation (Pozen,1980 and 1984; Lee,1985). The characteristics of the presenting symptoms most likely to be associated with a diagnosis of AMI appear to be:

Sudden onset of pain, duration of pain greater than 60 minutes, pressure or burning like character of the pain, precordial or left-sided position, previous history of ischaemic heart disease and age greater than 60 years (Pozen,1984; Lee,1989; Berger,1990; Karlson,1991). These characteristics can be formulated into a diagnostic algorithm, but even if combined with certain ECG criteria, the sensitivity and specificity of correct diagnosis are only 88% and 74% respectively, with a positive predictive value of 32% (Goldman,1988). This is just slightly better than the average performance of a selection of emergency room physicians. Even in the best hands, use of this diagnostic tool would result in a significant number of patients receiving inappropriate treatment (false-positives), and perhaps even more importantly, a large number of patients not receiving the correct therapy (false-negatives) (Lee,1989).

Although a number of diagnostic protocols have been described, they are not in widespread use and their applicability for use in the district as opposed to the teaching centres where they were formulated has been questioned (Young,1987). Their main use would appear to be excluding low-risk cases from admission to the CCU, rather than to positively differentiate between those patients with chest pain of cardiac aetiology who have sustained an AMI and so would benefit from the administration of thrombolysis, and those who have not. The fact that one group applied such a protocol to increase the number of patients discharged at 24 hours

after admission, as opposed to providing a firm diagnosis in the casualty department serves to illustrate this point (Weingarten,1989). Lee showed that a combination of the history, ECG changes and early enzyme estimations could separate patients at risk of serious complications at 12 rather than 24 hours after symptom onset (Lee,1991). Similarly, Gaspoz used a diagnostic protocol to decide which patients would be suitable for admission to an observation ward rather than the CCU, (those patients with a less than 10% chance of having AMI), the primary objective being to reduce in-patient management costs rather than identify those patients requiring thrombolytic therapy (Gaspoz,1991).

It can be appreciated from above that the nature of chest pain described in the casualty department is, overall, a poor indicator of the final (discharge) diagnosis. Patients with pain strongly suggestive of an acute myocardial infarction are often found to have a non-cardiac aetiology (Karlson,1991), and conversely, patients suspected of having no underlying acute cardiac pathology may be proven to have had an AMI in the subsequent days after admission (Goldman,1982). The fact that a number of computer-derived protocols have now been formulated but none appear to be in common use illustrates the difficulties of using a single symptom (albeit with a number of closely associated clinical features) to make a diagnosis of such importance. The need for a diagnostic parameter of far greater specificity is clearly apparent. The applicability of ECG changes in this regard will now be discussed.

1.3.3 ELECTROCARDIOGRAPHIC CHANGES ASSOCIATED WITH ACUTE MYOCARDIAL INFARCTION

The 12-lead electrocardiogram provides a considerable amount of information in the diagnosis of AMI; as indicated above it forms one third of the triad of AMI diagnosis. Its advantages are that it is easy to perform, inexpensive, reproducible and non-invasive. The potential for misinterpretation of the standard 12-lead ECG exists because:

a) it may not change with sufficient magnitude in response to changes in myocardial blood flow, b) alterations in flow to a given myocardial territory may not always result in the same electrocardiographic changes, and c) interpretation of the 12-lead ECG varies between different observers (Schweitzer,1990a).

It is accepted that certain changes in the resting 12-lead ECG are associated with AMI. These are as follows:

Q-WAVE AMI

i) The development of new or presumed new Q-waves of greater than 40 milliseconds (ms) duration in 2 or more anatomically contiguous leads by day 3 after the onset of chest pain (Lee,1985; Turi,1985; Boden,1989b).

ii) ST segment elevation ≥ 0.1 mV at 80 ms after the J point in 2 or more anatomically contiguous leads on the admission ECG (Turi,1985; Sharkey,1988).

iii) A loss of R wave amplitude of $>25\%$ in the precordial leads compared to the admission ECG (Lee,1985).

NON Q-WAVE AMI

i) The development of ST segment depression of ≥2mm at 80 milliseconds after the J point or

ii) ST segment elevation of ≥1mm amplitude and/or

iii) Associated T wave changes (inversion or pseudo-normalisation)

All of these changes being accompanied by a subsequent rise in certain serum enzyme concentrations, the ECG abnormalities persisting from the initial ECG to day 3 (Raunio,1979; Sharkey,1988; Schweitzer,1990b).

POSTERIOR AMI

i) The development of an R wave of ≥ 40 ms duration in lead V1, and an R:S wave ratio of ≥1 in lead V1 or V2 by day 3 (Boden,1989b; Schweitzer,1990a).

These changes can be incorporated into more detailed ECG classifications, for example: the Minnesota code (Blackbum,1969) and the World Health Organisation code (WHO,1959). These definitions are by and large accepted, but they have been in use for some considerable time and are recognised to have limitations. Generally, problems arise when the initial ECG recorded from a patient with a history suggestive of AMI does not fit into any one diagnostic category, or has old changes or features that make interpretation difficult, or does not show any changes at all.

A number of studies have been performed using these criteria to diagnose AMI from the admission ECG, AMI being confirmed by subsequent elevation of

creatine kinase-MB fraction. Rude et al assessed 3697 patients with suspected AMI and found a sensitivity and specificity for the ECG of 81 and 69% respectively (Rude,1983). It was noted that some changes were of more value than others. ST segment elevation with or without new Q-wave development diagnosed AMI correctly in over 80% of cases, whereas ST segment depression was accurate in just over 50%. Lee et al studied 7734 patients attending a casualty department with a diagnosis of presumed AMI, and revealed that by day 3, only 14% had ECG criteria for AMI (Lee,1989). However, the admission ECG was reported as showing definite changes of infarction in 8% of patients, and of this group only 76% actually had an AMI. He had previously studied 482 patients admitted with chest pain of greater than 30 minutes duration and an abnormal ECG, and found almost half to have no evidence of ongoing ischaemia or infarction (Lee,1985). In the same study he found that when used in conjunction with the site, character and associated features of chest pain, a single 12-lead ECG was of no additional value in helping to confirm or exclude the diagnosis of AMI. These results are lower than those seen in similar studies, but do indicate the problems associated with using a single ECG as the main arbiter of infarction.

In a large study evaluating over 7,000 patients presenting with chest pain presumed to be cardiac in nature, Karlson noted that of 4,690 patients admitted to hospital 921 had sustained an AMI (Karlson,1991). The initial ECG was categorised as indicating an obvious AMI in just 27% of these 921 patients. The main conclusion from the author was that a low suspicion of AMI on the presenting ECG results in a low probability of AMI by day 3 criteria, but the converse argument follows that if thrombolytic therapy was administered on ECG criteria alone, 73%

of patients would have been undertreated in this particular study.

Overall, a number of studies evaluating similar groups of patients have shown that for AMI, the diagnostic sensitivity of the admission ECG (where the ECG is abnormal) is 75-94%, but the specificity tends to be lower, of the order of 60-70% (Paton,1957; Bren,1987; Turi,1985).

There are a number of explanations to account for this inability of the ECG to correctly classify all patients at the time of presentation with suspected AMI. Firstly, a significant number of patients have persistent ECG abnormalities that do not permit diagnosis of infarction, for example, left bundle branch block (LBBB), previous changes of infarction, "strain" pattern of left ventricular hypertrophy etc. These have been identified in as many as 12% of patients presenting with suspected AMI (Willich,1987; Robalino,1989; Ohman,1990a). Although techniques such as QRST isointegral maps have been shown to be of some diagnostic benefit in these patients, the fact that it requires up to 87 unipolar electrodes and specialised software makes it impractical for routine use (Hayashi,1989). Inappropriate ECG changes (both ST segment elevation and depression), that may accompany conditions such as acute dissection of the aorta, can also result in an incorrect diagnosis (Satler,1984).

Secondly, an evolution in ECG changes following AMI is observed. Initially, an increase in R wave and T wave amplitude is seen, with associated ST segment elevation. This progresses to development of Q-waves, loss of R wave amplitude and T wave inversion (Von Essen,1985; Klainman,1987). The initial magnitude of these changes gives a guide to the final ECG appearance but is far from clear. The pattern of ECG changes at day 3 is relatively easy to discern, but as these changes

may not develop for up to 30 hours from the moment of infarction (Yusuf,1981), this makes interpretation of the ECG recorded on day 3 considerably easier than that recorded on day 1; especially when up to 20% of patients with AMI have been noted to have no discernible ECG changes on their admission ECG (Timmis,1990).

Thirdly, observer error can account for up to 4% of incorrect diagnoses from the admission ECG, usually dependent upon the seniority of the physician interpreting the ECG trace (Lee,1987a).

Some of these problems have been addressed by such workers as Greenberg, who has proposed a new diagnostic ECG code based on the region in which changes occur and their severity (Greenberg,1987). This produces a 4-point code for each patient and he states that this provides improved diagnostic and prognostic information although concedes that problems of interpretation still exist, and certain patients cannot be satisfactorily categorised, for example, those with a non Q-wave posterior AMI.

It would appear therefore that the term "myocardial infarction" implies that some degreee of myocardial necrosis has occurred, but does not indicate either the magnitude or the location of this. To expect an indirect recording such as a 12-lead ECG to show consistent features for all types of AMI is simplistic and inevitably this does not occur.

1.3.4 ELECTROCARDIOGRAPHIC DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION IN THE THROMBOLYTIC ERA

The importance of correctly diagnosing patients at the time of admission to hospital has been accentuated in light of the greatly increased use of thrombolytic therapy in the management of AMI. The clinical benefit of early administration of thrombolytic therapy, and the recognition that the prognostic profiles of QAMI and NQAMI differ, have made it necessary to delineate more precisely the occurrence and implications of acute ECG changes in the immediate post-infarct period.

1.3.4.1 ECG PREDICTION OF BENEFIT FROM THROMBOLYSIS

The evidence from numerous large trials of thrombolytic therapy in AMI suggests definite benefit for patients with ST segment elevation on the admission ECG (GISSI,1987; ISIS-2,1988; ASSET,1988; AIMS,1988). Von Essen et al showed that if the sum total of ST elevation was <2mV, this had a 100% sensitivity and 92% specificity for predicting sub-total coronary artery occlusion as confirmed at angiography (Von Essen,1985; Tanabe,1990).

Patients with no ECG changes on admission also seem to benefit from thrombolytic therapy, but those with ST segment depression or previous infarction appear to show no significant difference from the placebo group (ISIS-2,1988; Schweitzer,1990a), although Dewhurst showed that patients aged less than 75 years of age who sustained AMI and had only ST segment or "non-specific" ECG changes had an especially poor prognosis, and so argues that they require more aggressive management (Dewhurst,1991). A large number of patients with non-diagnostic ECG changes or changes that are difficult to interpret, such as "true" posterior infarction,

are thought to benefit from thrombolytic therapy (Benhorin,1990). With the admission ECG usually being the main criterion to determine who receives thrombolytic therapy, it follows that this latter group of patients in particular is liable to be undertreated.

In order to improve the current use of the ECG in the diagnosis of AMI and its influence in determining who receives thrombolytic therapy, certain authors have proposed that an increase in the number of changes on the admission ECG, (especially certain ST segment and T wave changes), that allow a patient to be identified as being in the high risk group for AMI should be permitted (Bren,1987; Spodick,1988; Lee,1989). This suggestion has derived principally from an increased understanding of the characteristics of QAMI and NQAMI and the realisation that previously held dogma is far from correct.

1.3.4.2 ECG PREDICTION OF TYPE OF INFARCTION

The correlation between ECG changes and anatomical site or pathological type of infarction has long been recognised as poor (Wilson,1944; Spodick,1983; Andre-Fouet,1989). The development of Q waves is still often taught as being secondary to a transmural AMI. However, as long ago as 1944, Q waves were shown to appear soon after experimental coronary artery occlusion and disappear when the ischaemic stimulus was removed, i.e. they were transient and not necessarily synonymous with myocardial necrosis (Bayley,1944). This is supported by the observation at necropsy that they signified a prior AMI in just over 50% of cases when inferior or precordial (Horan,1971). Post-mortem studies from as early as 1934 have shown that patients with QAMI do not always have transmural

infarction (Barnes,1935; Erhardt,1974; Savage,1977; Raunio,1979), and conversely patients with NQAMI may have transmural infarction (Myers,1949; Horan,1971). Although the validity of post-mortem studies has been questioned because they inevitably use a select group of patients, usually with more serious multi-vessel disease (Dwyer,1990), the consistency of findings of these various independent studies would appear to confirm their validity.

The time taken for Q-waves to develop is related to coronary artery anatomy and also can help to predict prognosis. If the time to Q-wave development is greater than 3 hours there is a far greater chance of a significant collateral circulation being present and these patients have the same early prognosis as those with NQAMI (Eisenberg,1991). Initial ST elevation is not a good predictor of who will proceed to develop Q-waves on the ECG (Boden,1989b); in addition, Q-waves may be transient. They have been reported to disappear from the resting 12-lead ECG in 10-20% of patients over 1-2 years from the date of infarction (Coll,1988).

A number of studies have shown that up to 50% of infarcts labelled as NQAMI at day 3 have $\geq$1mm ST segment elevation at the time of admission (Turi,1985; Huey,1987; Boden,1988; Schweitzer,1990a). To explain this, it has been postulated that ST segment elevation is a sign of transmural myocardial ischaemia, but does not necessarily imply progression to transmural infarction. This is confirmed by ECG recording during PTCA (Berry,1989).

1.3.4.3 NON-CLASSICAL ECG CHANGES DURING AMI

ST segment depression and/or T wave changes may occur secondary to myocardial infarction, and not only myocardial ischaemia as was previously thought.

Boden et al discovered that 15% of patients presenting with only ST segment and T wave changes proceeded to develop Q-waves by day 3 (Boden,1989b). In the Diltiazem Re-infarction Study (Boden,1987), 9.2% of patients included in the study had isolated horizontal ST segment depression with upright T waves on the admission ECG. Of this group of patients, 40% had a proven posterior AMI by the third day after admission, according to ECG and enzyme criteria. Those patients with downsloping ST segment depression and inverted T waves did not develop changes compatible with posterior AMI, but did show a rise in cardiac enzymes and so were labelled as having a "true" non Q-wave anterior myocardial infarction. In another study, up to 50% of patients presenting with ST segment depression had sustained an AMI (Lee,1993).

Angiographic studies have demonstrated that occlusion of the left circumflex artery (LCA) or right coronary artery (RCA) may be associated with ST depression rather than ST elevation (Boden,1987; Mamby,1987). In patients with AMI secondary to occlusion of the LCA, Huey et al found ST segment elevation in just 48%, and no significant ECG changes in 38% (Huey,1988). This contrasts significantly to RCA and LAD occlusion where 71% and 72% respectively had ST segment elevation on the admission ECG and is supported by ECG recording during PTCA, where occlusion of the LAD and RCA produced ST elevation in 84 and 92% of patients respectively, but LCA occlusion only 32% (Berry,1989). Landzberg found that only 27% of patients with LCA obstruction developed Q-waves (Landzberg,1991).

This observation may help to explain why in studies where ST elevation was the main entry criterion to receiving thrombolytic therapy, the LCA was the infarct

related artery in just 8-17% of cases (TIMI, 1985; Blanke,1984b; Huey,1988). Administering thrombolytic therapy only when ST segment elevation occurred would have prevented it being given to 50% and 25% of patients with AMI secondary to occlusion of the LCA and RCA respectively (Blanke,1984b). Overall, ST segment elevation appears to be 91% specific but only 46% sensitive for AMI (Bren,1987).

1.3.4.4 ECG CHANGES AND SITE OF INFARCTION

The association between the leads in which ECG changes occur, and the infarct related artery, and so the actual area of myocardium affected, is also variable (Stadius,1985; Huey,1988; Dwyer,1990). In general, the correlation between occlusion of the LAD and anterior myocardial infarction is good (Savage,1977), with lead V2 being the best for detecting associated ECG changes (Schweitzer,1990a). Apical AMI was best detected by the development of new Q-waves in leads V1-V4 and an associated loss of R waves, but this was only accurate for 39% of cases (Giannuzzi,1989).

Occlusion of the RCA was seen to give rise to ST segment elevation with or without new Q-wave development in leads III and aVF in up to 90% of cases (Blanke,1984b; Huey,1988). Birnbaum et al regard lead aVL as the best for detecting inferior myocardial infarction (Birnbaum,1993). Right ventricular infarction (secondary to proximal RCA occlusion) is often missed because lead V_4R is not recorded . This lead has been reported to have 100% sensitivity and 82% specificity for right ventricular infarction (Cohn,1974; Mukhardi,1984; Robalino,1989). Occlusion of the LCA produces ST segment elevation in leads I, aVL, V5 and V6 in only 50% of cases (Blanke,1984b; Huey,1988), with Q-waves

developing in only 20% of patients (Schweitzer,1990a). No ECG changes were reported in 38% of patients with AMI secondary to LCA obstruction (Huey,1988). LCA occclusion can result in inferior AMI, and is more likely to give rise to a "true" posterior or isolated lateral infarct (Mamby,1987). Differentiation of RCA from LCA occlusion from a single ECG is recognised as difficult (Dwyer,1990; Fuchs,1982). Overall, non-specific ECG findings are more likely to be secondary to pathology in the LCA than in the LAD or RCA (Blanke,1984b).

These variations in ECG changes following specific coronary artery occlusions are mostly thought to be secondary to anatomical variation, for example, the LAD can terminate at the apex of the left ventricle or it can supply up to 1/3 of the inferior surface of the LV (Dwyer,1990). Similarly, the LCA or RCA can be "dominant", that is, give rise to the posterior descending artery, and so supply varying amounts of myocardium.

1.3.4.5 CONCOMITANT ST SEGMENT ELEVATION AND DEPRESSION IN ACUTE MYOCARDIAL INFARCTION

Isolated changes of ST segment elevation or depression in specific groups of leads on the 12-lead ECG, specifically, leads II, III and aVF (inferior leads), leads V1-V4 (anterior) and leads I, aVL, V5 and V6 (lateral), are recognised as being secondary to alterations in myocardial blood supply to relatively discrete areas of myocardium (Lembo,1986; Boden,1989a).

Combinations of ST segment elevation and depression in different lead groups are seen. Up to 72% of patients with ST segment elevation inferiorly may have ST depression anteriorly, and up to 57% of patients with ST elevation

anteriorly may have ST depression inferiorly (Raunio,1979; Katz,1986; Yousif,1989). A number of explanations have been proposed to explain these so-called "remote changes" (Schuster,1981; Gibson,1982; Camara,1983; Ferguson,1984):

i) That differing degrees of infarction have taken place in more than one area of myocardium, for example, a QAMI inferiorly and a NQAMI anteriorly.

ii) That extension of the infarct has occurred posteriorly.

iii) That coronary artery flow is affected by the altered haemodynamics accompanying myocardial infarction. This implies that atheromatous disease is present in additional vessels to the infarct related artery and that myocardial ischaemia is produced with ST segment depression being manifest on the 12-lead ECG.

iv) That "benign" reciprocal changes mirror the electrical changes taking place elsewhere (Timmis,1993).

This last view (iv), is the least popular because these ECG changes often occur in areas of the heart that are not truly electrically opposite. For example, inferior and anterior planes are at 90,° not 180° to one another (Boden,1989a). The fact that these changes are not always seen (which would be expected if they were simply an electrical phenomenon), and the fact that patients with such remote changes have a significantly worse in-hospital prognosis than patients without such changes does indeed suggest that they signify some additional abnormality of myocardial perfusion.

The repeated observation that patients with ST segment depression in

addition to ST segment elevation do less well than patients with just one of these ECG abnormalities suggests that more vessels are threatened, probably collaterals, which results in a greater incidence of subsequent ischaemic events (Boden,1989b). Up to 2/3 of patients with remote ECG changes were found to have a stenosis of at least 70% in the artery supplying that area (Yousif,1989). Yousif also demonstrated that remote ECG changes after AMI were shown to be as good as sub-maximal exercise testing at predicting multi-vessel disease. This finding is contradicted by the study of Odemuyiwa, who found that remote ST segment depression on the admission ECG did not predict patients with atheromatous disease in the vessel supplying that region (for example the LAD in anterior changes) (Odewuyiwa,1985).

Shah et al first described the clinical and prognostic implications of precordial ST segment depression in association with inferior ST segment elevation (Shah,1980). In a study comparing patients with first myocardial infarction who had ST segment elevation inferiorly and ST segment depression in leads V1-V4, patients in whom precordial ST segment depression persisted after 24 hours had a significantly worse outcome in terms of morbidity, mortality and LV ejection fraction as documented by clinical, haemodynamic and non-invasive investigations, than those in whom it had resolved (Lembo,1986). This finding was confirmed in similar groups of patients (Bates,1990; Lee,1993). Bates concluded that the initial magnitude of precordial ST segment depression in patients with ST segment elevation inferiorly helps to identify an important subset of patients with first AMI who are at added risk. He also advocates using thrombolytic therapy for those patients with ST segment elevation inferiorly only if they have had pain for less than

3 hours or if they have ST segment depression anteriorly (Bates,1988).

One study which contradicts the view that ST segment elevation and remote ST depression is of increased significance to ST segment elevation alone is that by Camara et al which identified this particular pattern of ST changes in 25 patients (Camara,1983). They noted an absence of any correlation between wall motion abnormalities and resolution of ST segment changes and proposed that this ST segment abnormality did not indicate underlying myocardial dysfunction because this change was so common and it could not realistically predict an important subset of patients.

The general concensus however seems to be that it is persistence of these changes beyond 24 hours that is important, and that because patients in whom this occurs have a worse prognosis, an additional insult to the myocardium is likely to have occurred.

1.3.4.6 DYNAMIC ST SEGMENT CHANGES AND ACUTE MYOCARDIAL INFARCTION

ST segment changes in response to alterations in myocardial blood flow have been recognised for a number of years (Eyster,1939). It was by using direct current coupled amplifiers to record both epicardial and endocardial waveforms in dogs that the pathophysiological changes responsible for these variations in the cardiac action potential were identified (Samson,1960; Vincent,1977). Two main effects of complete or partial occlusion were noted:

i) A variation in resting membrane potential (incraese or decrease) in the region of myocardium with altered blood flow,

ii) An alteration in the transmembrane potential waveform (in terms of amplitude, duration and slope of phase 2 of the action potential), and its time of onset (delayed activation and repolarisation).

During any alteration in myocardial blood flow subendocardial tissue is first affected. ST segment and T wave changes therefore represent a diastolic injury current, because coronary blood flow occurs principally in diastole.

On the surface ECG, loss of resting membrane potential results in the true baseline (TQ segment) being depressed, this is the most common consequence of impaired myocardial flow. If the baseline remains in the same position on the ECG recording, then this gives the impression of ST segment elevation. Conversely, an upwards shift in this TQ segment baseline gives the impression of ST segment depression.

Changes to the action potential waveform morphology alter the shape of the ST segment. These changes occurred less frequently than those to the TQ segment, and were affected more by variation in heart rate than by alterations in blood flow. T wave morphology also varies during myocardial ischaemia, principally by a change in polarity.

1.3.4.7 ECG CHANGES AND CORONARY ARTERY REPERFUSION

ST segment monitoring immediately after myocardial infarction has been advocated as a non-invasive marker of coronary artery patency. A large number of studies have used various methods to assess coronary artery reperfusion. These have all been validated with coronary angiography, although the timing of angiographic

assessment in relation to the observed ECG changes is not identical for all studies. That is, some studies relate ECG changes to coronary angiograms performed soon after the administration of thrombolysis, for example, Hogg et al (Hogg,1988), whereas other studies performed angiography considerably later, some 6 days after thrombolysis, (Richardson,1988). Considering the fact that coronary reperfusion is a dynamic, rapidly changing process that is often accompanied by intermittent or sustained reocclusion, the precise relation between ECG changes and reperfusion status is difficult to quantify, although from the GUSTO angiographic substudy it does appear that early, complete reperfusion is of considerable prognostic significance (GUSTO-I,1993).

Saran et al showed that a reduction in the degree of ST segment elevation of greater than 25% within 3 hours after the administration of thrombolytic therapy was compatible with either a patent artery, or preservation of left ventricular function (Saran,1990). A fractional change (reduction of ST segment elevation) of greater than 0.5 within 2 hours of intra-coronary, or 3 hours of intra-venous, streptokinase therapy was also corresponded to coronary artery patency (Lee,1989)

Hogg et al measured the sum of ST segment elevation in all the affected leads of patients with anterior AMI, on admission and at 48 hours (Hogg,1989). They demonstrated a significant reduction in the degree of ST elevation at 48 hours in those patients who received thrombolytic therapy compared to those not being thrombolysed. However, the confidence limits identified did not allow any individual patient to be categorised with certainty.

Using 50% resolution of the amount of ST segment elevation on the admission ECG as a marker of reperfusion, Hohnloser et al found this to give a

sensitivity and specificity of 60% and 95% respectively (Hohnloser,1991). Clemmensen found that a 20% resolution of ST elevation measured at the J point gave a sensitivity and specificity of 88% and 80% (Clemmensen,1990b), Baskin et al found a sensitivity of 62% for this amount of ST segment resolution (Baskin,1993). An alternative approach was employed by Krucoff (Krucoff,1989), who used the time to achieve a steady state following thrombolytic therapy as a marker of reperfusion. This was defined as the time from administration of thrombolytic therapy to the time when the ST segment level was equally or less deviated than the quiescent level (inevitably this incorporates a retrospective component to the analysis). When this steady state was achieved in less than 100 minutes this gave a sensitivity and specificity for reperfusion of 89% and 82% respectively. The occurrence of reperfusion arrhythmias or time to resolution of chest pain was found to be of no use in identifying patients who had satisfactorily reperfused the infarct related artery. (Saran et al,1990; Krucoff,1989), although Shah observed that the sudden development of sinus bradycardia, or the onset of an idioventricular rhythm was associated with reperfusion (Shah,1993).

It is increasingly recognised that ST segment changes in the immediate post AMI period, either with or without the administration of thrombolysis are dynamic. Krucoff et al monitored ST segments continuously and found cyclical changes in several patients. They concluded, by measuring other clinical variables, that where ST segments fluctuated, the "quality" of reperfusion was less than when this did not take place (Krucoff,1993). Kwon et el identified a group of patients in whom ST segment re-elevation occurred after resolution. This group had reduced arterial patency, and more severe residual stenoses than patients without recurrent ST

segemnt elevation (Kwon,1991). Miida et al quantified this by reporting that if the sum of ST segment re-elevation was $\geq$0.5mV in leads V1-V6, then a greater amount of myocardium had been damaged prior to thrombolysis than if this re-elevation was not present (Miida,1994). This was supported by Kondo et al who found a similar group of patients to have larger infarcts and a worse prognosis (Kondo,1993). Simoons et al acted upon this observation and gave repeat thrombolysis (tPA) to patients with re-elevation noting an improved outcome than when this was not done (Simoons,1993). Only Schechter and colleagues appear to regard ST segment re-elevation as a favourable finding. They found that patients with anterior AMI had improved left ventricular function if ST segment re-elevation was seen. There was neither a positive or negative effect on patients with either inferior or posterior AMI (Shechter,1992)

Other components of the ECG recording have been evaluated for identifying reperfusion. Tanabe et al measured the sum of ST segment elevation (ΣST) and the sum of R wave amplitude (ΣR) in a number of patients with anterior AMI (Tanabe,1990). All patients categorised as having successful reperfusion had $\Sigma R/\Sigma ST > 2.5$ or $\Sigma ST < 2mV$. Using a 48-lead mapping system, unsuccessful thrombolysis or re-occlusion was demonstrated by a decrease in total R-wave amplitude, or the number of leads with no R-waves present (Von Essen,1985).

Late potentials are high frequency, low amplitude signals not usually detected by routine electrocardiography. They appear to identify patients at risk of future cardiac events following AMI. When comparing patients with and without coronary artery reperfusion, a significant difference was identified between the 2 groups, but there was a far greater overlap than that seen with ST segment shifts, and

consequently they have no clinical application in this area (Tranchesi,1990).

Significant ECG differences can be identified between groups of patients who have and have not received thrombolytic therapy. In the TAMI 7 trial, of 11 parameters assessed as non-invasive markers of coronary artery reperfusion, ST segment recovery was the only one with any useful discriminatory power (Krucoff,1991). Other authors have reviewed this subject and comment that of the many methods of interpretation of ST segment changes considered, although overall there may be differences between the 2 groups, these measurements have no clinical applicability to individual patients (Bren,1987; Hillis,1990; Boden,1991). This finding was confirmed by Zabel et al who found continuous ST segment monitoring to have similar sensitivity, specificity, positive and negative predictive value to reporting ST segment changes on 2 ECGs taken 2 hours apart, these being of the order of 77%, 74%, 89% and 50% respectively (Zabel,1992).

An overview of the studies of early patency assessment using serial ECG monitoring techniques deduced that rapid resolution of ST-segment elevation of at least 50% is probably the best criterion of early patency (Klootwijk,1993).

Although at present the application of ECG changes at an individual level is not completely valid, the possibility of combining one or more of the above techniques with biochemical or other markers of reperfusion exists. This may allow application of these techniques to identify more accurately individual patients who have, or have not, achieved reperfusion of the infarct related artery.

1.3.5 SUMMARY

Most studies utilising the ECG as the main entry criterion for receiving

thrombolytic therapy exclude a large number of patients in clinical practice who often provide the most difficult cases to manage. It is clear that there is usually no difficulty in diagnosing AMI from an ECG with "classical" ST elevation, but problems of correct diagnosis arise for those patients with a history suggestive of AMI, but who have a non-diagnostic ECG, that is, non-classical changes, and/or a past history of one or more of the following which complicates matters further: Previous AMI, past history of valvular and/or congenital heart disease, cardiomyopathy, previous coronary artery bypass grafting (CABG) or the presence of left or right ventricular hypertrophy (LVH or RVH), permanent pacemaker (PPM), left or right bundle branch block (LBBB or RBBB) or drugs that affect the resting ECG, such as digoxin. All too often, these are patients that are more likely to develop infarct extension or have recurrent AMI (especially occurring soon after the index MI) and they constitute a significant proportion of patients presenting with symptoms suggestive of acute infarction (Marmor,1982; Lembo,1986).

It can be appreciated that changes to a particular group of leads on the 12-lead ECG should not always be regarded as accurately signifying changes in a particular region of myocardium and, even less so, flow in a particular coronary artery. ST segment depression does not always denote myocardial ischaemia and lack of inversion of T waves may actually represent an event of greater importance than actual inversion of T waves, especially when accompanied by other changes e.g. anterior ST segment depression. Furthermore, it is not possible to predict from the admission ECG which patients will develop Q-waves by day 3.

The need for additional means to detect changes in myocardial flow is apparent. Biochemical tests used in this role will now be considered.

1.4 BIOCHEMICAL MARKERS OF ACUTE MYOCARDIAL INFARCTION

1.4.1 INTRODUCTION

One of the earliest histological manifestations of myocardial infarction is disruption of the sarcolemmal membrane (Jennings,1965). Following disruption, intracellular proteins are able to escape into the circulation and a number of these have been assessed as markers of myocardial injury.

Historically, such proteins were used simply as confirmation of the diagnosis of AMI, but more recently, the need to determine rapidly whether reperfusion of the infarct related artery has occurred has led to a search for new markers and renewed interpretation of previously identified ones. These will now be discussed.

1.4.2 ASPARTATE AMINOTRANSFERASE

A rise in serum concentration of aspartate aminotransferase (AST) following AMI was first reported in 1954 (Karmen,1954). Peak AST concentration occurs 22 $\pm$ 7 hours post-AMI (Muller-Hansen,1989). Until relatively recently AST was in widespread use in the diagnosis of AMI, but currently it is regarded to be of most use when diagnosing AMI 48-72 hours after it has actually occurred (Rotenberg,1989; Pappas,1989); it has no effective role within the recognised time window of effective thrombolytic therapy AMI (Lott,1989a).

1.4.3 LACTATE DEHYDROGENASE

Serum concentrations of lactate dehydrogenase (LDH) have been known to increase following AMI for some considerable time. Total LDH can be of value in diagnosing AMI up to 72 hours after the onset of chest pain (Rotenberg,1989). Peak

LDH levels are seen 34±12 hours following AMI (Muller-Hansen,1989), and consequently in the first few hours following AMI, LDH is of no diagnostic value (Collinson,1988; Lott,1989a).

Various isoenzymes of LDH can be measured. For diagnosis of AMI, of most value is the isoenzyme LDH-1 (alpha-hydroxybutyrate), and the ratio LDH-1/total LDH (Collinson,1988).

As for AST, LDH has no role to play in the diagnosis of AMI within the first few hours after the onset of symptoms, but for patients presenting late, that is greater than 48 hours from the onset of chest pain, LDH-1 was, until recently, the enzyme of choice for making a retrospective diagnosis of AMI (Roberts,1988).

1.4.4 CREATINE KINASE

Creatine kinase (CK) release as a diagnostic marker of AMI was first described by Hughes in 1962. CK concentrations peak at 20 hours after the onset of symptoms, but, more importantly, levels are seen to rise within a few hours in some patients (Hughes,1962).

The time to measure CK can be reduced by using an analyser at the bedside rather than requiring laboratory analysis. Gibb evaluated a benchtop reflectance photometer and found its performance satisfactory when compared to laboratory analysis (Gibb,1985), as did Downie et al using a bedside assay (Downie,1993). A CK result was available from a plasma sample within 4 minutes and gave a result up to 10 hours earlier than that provided by the "routine" service. This illustrates the usefulness of such an instrument, but does not eliminate the problems of CK measurement in the diagnosis of AMI.

The main disadvantage of CK as a marker of AMI is the fact that it is not specific to the myocardium, and therefore a rise in serum concentration can occur in a number of pathological conditions and hence the specificity of the test is reduced. Lee et al found that a single measurement of CK gave a positive diagnosis in only 25% of patients within 4 hours from the onset of symptoms, and an overall frequency of false-positives of 20% (Lee,1987b). The lack of reliability of a single measurement is confirmed by a number of other authors (Irvin,1980; Doran,1990).

Creatine kinase is composed of two subunits. One, subunit M, is so called because it derives principally from skeletal muscle and forms the isoenzyme CK-MM. The other, subunit B, is most abundant in the brain and forms CK-BB. The hybrid form, CK-MB is found principally in the heart, where it comprises about 15% of total myocardial CK activity. As well as the three isoenzymes, a number of isoforms have also been identified, 4 for CK-MM and 2 for CK-MB (Lott,1989b).

Kontinnen and Somer in 1972 first described the release of CK isoenzymes following AMI (Konttinen,1972). They showed that the majority of enzyme released is CK-MM, and that CK-MB as a percentage of total CK ranged from 0-28%. In most patients a rise in CK-MB can be detected within 6 hours from the onset of chest pain, a peak value is seen in 10-36 hours and the levels return to normal within 48-72 hours (Kimmelsteil,1989).

The difference between the diagnostic abilities of CK and CK-MB is illustrated in a study by Clyne et al (Clyne,1989). Patients with a normal total CK but elevated CK-MB had a similar clinical course to patients with raised CK and CK-MB, and a significantly worse course than patients with neither a CK or CK-MB rise. He proposed that for accurate diagnosis of AMI, CK-MB must be

measured. Lott confirmed that CK-MB was of greater diagnostic value than CK (Lott,1989a) and the specificity of CK-MB was demonstrated by the fact that 2 sequential negative results had a negative predictive value for AMI of 98% (Kimmelsteil,1989; Roberts,1988). This finding is not universal, since Kallner et al in a comparison of the diagnostic abilities of total CK with CK-MB revealed no significant differences between the two up to 12 hours from the onset of symptoms (Kallner,1989).

The consensus at present is that serial analysis of CK-MB is the most sensitive, specific and cost effective means of diagnosing AMI. It is regarded as the gold standard of AMI diagnosis (Lott,1989a). Roberts advocates that for the majority of patients there is no need to measure other enzymes, and adds that total CK and CK-MB should be measured every 4-6 hours (Roberts,1975). The accepted criteria for diagnosis are CK-MB activity >5% total CK activity, 12-36 hours after the onset of symptoms (Vijan,1991).

Wu et al compared the analytical performance of three immunoassays used to measure CK-MB rapidly, including Hybritech's "ICON QSR CK-MB" (a two-site immunoassay utilising immunoconcentration) (Wu,1989). Performance criteria included precision, analytical sensitivity, sample stability. They concluded that there was no significant difference in the analytical characteristics of these assays but felt that the ICON method was probably best suited to a rapid diagnostic role, since only a relatively inexpensive reader is required in addition to the individual test cups. The limitations of the assay range, namely a lower and upper cut-off of 2 and 50 ng/ml respectively, did not detract from its potential value in the diagnostic setting. These findings were supported in a similar study in 195 patients presenting with

chest pain (Collins,1993).

Mair et al demonstrated that CK-MB mass concentrations are elevated sooner following AMI than CK or CK-MB activity levels. They suggest that in the post-thrombolytic era, mass methods of detection should be employed wherever possible (Mair,1991b). The improved specificity of a CK-MB mass assay in comparison to a CK-MB activity assay in patients following PTCA (without evidence of co-existent AMI) was demonstrated by Hunt et al (Hunt,1991), who showed 9% of patients to have a rise in CK-MB activity but not in CK-MB mass concentration. The validity of this study being supporteded by the observation that Troponin-I levels did not increase, so confirming that myocardial infarction was unlikely to have occurred.

An alternative approach to using absolute values of CK and its isoenzymes in making an early diagnosis of AMI is to exploit the fact that a rise in concentration rather than a particular cut off diagnostic value be employed. Collinson et al evaluated the slope of the log concentration of a number of enzymes against time, and found that a slope log CK /hour >0.015 for samples taken on admission, 6 and 12 hours after the onset of chest pain was both highly sensitive and specific for the diagnosis of AMI (Collinson,1988). This finding was confirmed by Mullen, who found all 16 patients with AMI to have a log CK slope of >0.015 from samples taken 4 to 16 hours after the onset of chest pain (Mullen,1990). Collinson has subsequently shown that log slopes of CK-MB activity or concentration did not yield any further diagnostic information to those of log slope CK/hour (Collinson,1989). He advocates that this method could be used at the bedside, but at present it requires the rapid laboratory analysis of more than one blood sample for CK as soon as the sample reaches the laboratory, and limits the

time to diagnosis to 12 hours after the onset of chest pain. Vijan et al using a similar method of log CK slope/hour found that 22% of patients with AMI were not diagnosed by this method (Vijan,1991), although no patients without AMI gave a positive result; the specificity being 100%, and the sensitivity 78%. It is also generally agreed that this technique works best for a low initial CK level, if the first CK-MB is high, the chance of the second level being of sufficient magnitude to produce a log slope/h >0.015 is reduced (DuFour,1989). For this reason, it has been suggested that a diagnostic cut-off level be decided upon and used to determine whether infarction has occurred (Leung,1991). LaGrenade studied 107 patients with AMI, and was able to make a correct diagnosis by measuring CK-MB levels on admission and 8 hours later (LaGrenade,1987). However, she used an electrophoretic or chromatographic method to analyse the samples and so the time delay to diagnosis on a fresh sample would be even greater.

Despite the fact that CK-MB is regarded as the gold standard of AMI diagnosis, it is fallible (Lott,1989b; Bakker,1993). This is because it is not entirely cardio-specific and hence other pathologies can result in a release of CK-MB from a non-cardiac source. The false-positive rate can be as high as 15% (Lee,1986), especially when the total CK concentration is high for example following trauma or surgery. In such situations, total CK-MB may be elevated, but the percentage of CK-MB as a fraction of total CK is usually reduced. Thompson studied 146 patients with a high total CK (>1000 U/L) and found that a cutoff of CK-MB >15 U/L, and a CK-MB proportion of the total CK of $>2\%$ gave optimum results for diagnosis of AMI (Thompson,1988). The ratio of CK-MB/CK gave a better specificity than absolute values of CK-MB alone, but resulted in a significant loss

of sensitivity. Even with these improved criteria a number of false-positive diagnoses would have been made.

The significance of a minimally elevated CK-MB concentration has also been called into question. White et al defined positive diagnosis of AMI as a CK-MB >25 IU/L, and a negative diagnosis as 0 IU/L (White,1985). Those patients with a CK-MB of 1-24 IU/L constituted a so-called intermediate group. Initially the mortality in the intermediate group equalled that of the positive group, but patients with chest pain alone in the intermediate group had a mortality of 0%, whereas patients with severe medical problems had a mortality of 33%. He suggested that in patients with results in this indeterminate range, other factors should be considered before deciding upon the significance of a minimally elevated CK-MB concentration, that is, extra-cardiac sources of CK-MB may be responsible.

At present, CK-MB is the best diagnostic marker of AMI due to the fact that it is relatively cardio-specific, its release kinetics following AMI allow relatively rapid identification soon after the onset of infarction, and because it has been in widespread use for a number of years, a large number of assay methods for accurate and reliable measurement have been developed (Bakker,1993).

As well as providing information to diagnose AMI, a number of parameters of CK-MB, and CK, release have been examined, principally in relation to identifying reperfusion of the infarct related artery and differentiation of QAMI and NQAMI.

1.4.4.1 EFFECT OF REPERFUSION ON SERUM CK-MB CONCENTRATIONS

Animal studies show that a rapid release of enzymes occurs following reperfusion of infarcted myocardium (van der Veen,1990). Comparing transient and persistent occlusion of the left circumflex coronary artery in dogs, Roe showed that following transient occlusion of 2 hours, maximum enzyme release occurred 15 minutes after release of the obstruction, whereas in dogs with persistent obstruction, peak levels were seen after 9 hours (Roe,1975). A study in rats gave similar results (Matsui,1989), and generally, the kinetics of myocardial marker protein appearance in the plasma compartment depends on perfusion of the infarcted area (Shell,1983). This phenomenon has been applied in a number of studies in man.

Nidorf compared early peaking of creatine kinase to reperfusion detected angiographically (Nidorf,1988). Initially during a pilot study, CK levels were measured 6 hours after the onset of symptoms, (immediately after administration of thrombolytic therapy), and at 12 hours. If the rate of rise of CK concentration was $\geq 7\%$ peak concentration per hour, this gave a 90% sensitivity and 76% specificity for coronary artery patency. Norris et al also use peak concentration, arguing that if the concentration of CK 3 hours after thrombolysis was $\geq 20\%$ peak concentration then reperfusion was likely to be present (Norris,1993). The obvious limitation here is that information relating to patency is available a minimum of 6-7 hours after thrombolysis, and this also implies CK analysis is performed almost immediately.

In a comparison of patients who received intra-coronary streptokinase with those who did not, Blanke found that the time to peak CK was less in the group receiving thrombolytic therapy; reperfusion was documented angiographically in these patients (Blanke,1984a). Two similar studies confirmed this observation

(Shah,1993; Ohman,1993). In patients with AMI, those individuals who have undergone successful reperfusion have peak CK-MB values about 10 hours after the onset of symptoms. In comparison, patients whose infarct related artery remains occluded show this peak at about 20 hours (Hackworthy,1988; Zwann,1988).

Lewis et al showed that in the first 150 minutes following thrombolytic therapy, patients with successful reperfusion had higher actual and relative increases of CK and CK-MB than patients in whom reperfusion did not occur (Lewis,1988). The authors noted that although the results for relative rate of enzyme rise were the most accurate, these required a knowledge of the peak value, which by definition, is not available until later. They suggested that using the absolute rates of rise is sufficient to make an informed pronouncement upon the state of arterial perfusion; principally whether TIMI grade 3 flow has been restored. Grande et al also calculated that if the rate constant for increase in concentration of CK was greater than 0.185 there was a strong likelihood of reperfusion (Grande,1991). More contentiously, they proposed that if the first sample was analysed prior to thrombolysis, then patients with spontaneous recanalisation can be identified (by the magnitude of the initial marker concentration) and a relatively conservative approach taken, with the converse approach for those patients in whom reperfusion does not appear to have occurred.

Other aspects of the pathology of AMI have also been considered with regard to marker serum profiles. Clemmensen et al compared patients treated with intravenous streptokinase or placebo. They found that in the treated group CK-MB appeared more rapidly, and by using ECG estimates of infarct size from the admission and day 3 ECG's implied that up to 60% of patients in the treated group

had demonstrated myocardial salvage (Clemmensen,1990a).

Ong et al performed coronary angiography soon after thrombolysis. They found that the rate of appearance of CK-MB was related to the severity of the residual stenosis, an infarct related artery stenosis of 1.2mm being the flow limiting lesion (Ong,1991).

Bosker et al compared the time to peak values, and release rates of CK and LDH-1 and found the accuracy for each of these 4 tests to be of the order of 70-89% for detecting reperfusion as demonstrated by angiography (Bosker,1991). The kinetics of LDH-1 release do not lend themselves to this function, and although a distinct cut off level did not exist between the 2 groups, a significant difference between them for time to peak CK and rate of rise of CK was observed. Bosker therefore appears to have highlighted the problem reported by a number of workers who feel that non-invasive detection of coronary artery reperfusion can distinguish between the 2 groups of patients involved, but that at an individual level this is not precise. Only with the incorporation of more markers or refining the technique and timing of measurement will this distinction become clearer.

1.4.4.2 COMBINING BIOCHEMICAL AND ECG CRITERIA IN THE DIAGNOSIS OF REPERFUSION

Hohnloser et al compared the individual and combined predictive abilities of a number of markers of reperfusion. They found that individually, the time to peak CK and 50% resolution of ST-segment elevation were moderately sensitive, highly specific, strongly positive predictors, but relatively weak negative predictors of coronary artery patency (Hohnloser,1991). A combined analysis of these 2 markers

produced a sensitivity of 100%, a specificity of 90%, positive predictive value of 97% and negative predictive value of 100%. The presence of reperfusion arrhythmias and/or resolution of ischaemic chest pain did not have any worthwhile contribution to make to the diagnostic process.

1.4.4.3 DIFFERENCES BETWEEN Q-WAVE AND NON Q-WAVE MYOCARDIAL INFARCTION

A difference in enzyme release kinetics has been observed between QAMI and NQAMI. Shell et al examined changes in CK-MB activity within the first 6 hours after admission. They found that in QAMI, only 72% of patients had a raised CK-MB level, compared to 100% of patients with NQAMI (Shell,1981). Of the 28% with QAMI and no detectable rise within 6 hours, it took until 12 hours after the onset of symptoms before a rise was detectable in all patients. Conversely, some of the patients in the NQAMI group had a detectable rise by 2 hours. A difference in the time to peak CK-MB was noted for patients with and without definite evidence for AMI on their admission ECG (Sharkey,1988). None of these patients received thrombolytic therapy. Those with a non-diagnostic ECG had an earlier but smaller peak than those with a diagnostic ECG. The majority of patients in the diagnostic group (16/21) had QAMI, whereas the majority in the non-diagnostic group had NQAMI (12/13). These findings are not surprising, but still show that up to 8% of patients proceeded to QAMI despite presenting with non-specific ECG changes.

Cox et al compared patients with early (less than 15 hours) and late (greater than 15 hours) time to peak CK-MB. They discovered a number of differences

between these 2 groups of patients. The "early" group were older, had an increased incidence of congestive cardiac failure and arrhythmias, but most significantly, had a recurrent infarction rate and 4-year mortality far higher than those patients in the late group (Cox,1987).

Carpeggiani et al employed a multi-parametric approach to the diagnosis of NQAMI. They discovered that using conventional criteria of a CK-MB rise to twice its normal value only 60% of NQAMI were correctly diagnosed in comparison to a number of other methods aimed at identifying myocardial necrosis, for example, pyrophosphate scanning, microsphere perfusion scintigraphy and coronary angiography (Carpeggiani,1989). They concluded that total CK-MB release in NQAMI may not be sufficient to allow detection unless frequent sampling is performed, that is, that because of an earlier peak a rise in serum concentrations may not be detected. This view is supported by Blanke, who advocates caution with regard to measuring infarct size from cumulative CK or CK-MB release because of significantly different rates of enzyme appearance in patients with reperfusion (Blanke,1984a). He proposes that measurement of time to peak CK-MB, and absolute value of peak CK-MB are definite quantities, and these should be used for early stratification of patients and subsequent management of these two groups of patients. The observation that enzyme levels peak earlier in NQAMI than in QAMI could be explained by a "washout" phenomenon in the NQAMI group due to spontaneous recanalisation and restoration of flow to necrotic myocardium. An alternative explanation may be that the time of onset of symptoms is less clear in this group and so is underestimated. Certainly, it seems that patients with NQAMI have smaller infarcts and also show features of improved myocardial reperfusion in

comparison to patients with QAMI (Blanke,1984a; Sharkey,1988).

Total cumulative release of CK is indicative of infarct size and is an important prognostic indicator (Hackel,1984). Shah studied patients with inferior AMI and noted the additional presence or absence of ST-segment depression in the precordial leads (Shah,1980). Those patients with ST depression had a higher morbidity and mortality in the first few days following AMI and had higher peak CK levels, suggesting that this worse early course was secondary to increased infarct size.

1.4.5 CREATINE KINASE-MB BANDS

Two isoforms of CK-MB have been described, MB_1 and MB_2. They are measured by electrophoresis and calculating the ratio of the 2 subforms from the total CK-MB activity (Christenson,1989). Puleo et al measured actual values and the ratio of these subforms following AMI, and showed that MB_2 activity and the ratio MB_2/MB_1 began to increase 2 hours after the onset of symptoms (Puleo,1990). The first available plasma sample was abnormal for CK-MB subform criteria in 67% of patients and by conventional CK-MB assay in 27% of patients. Assay of subforms therefore provided a rapid and reliable diagnosis of AMI within 4-6 hours after the onset of symptoms, 6 hours before conventional CK-MB assays gave a definite result.

Christenson et al measured CK-MB isoforms following myocardial reperfusion with intravenous thrombolytic therapy; recanalisation was demonstrated by immediate coronary angiography (TIMI 3) (Christenson,1989). They showed that in comparison to patients without reperfusion, CK-MB_2 peaked earlier than CK-MB

(4.5-8.0 h v 5.75-10.0). A similar association was seen for CK-MM_3. Although the MB_2/MB_1 ratio peaked at 0.75-2.25 hours, this did not distinguish between patients with and without reperfusion (Christenson,1992). The release of tissue specific CK isoforms does appear to be related to coronary flow, but isoform ratios have a relatively weak association, although they may be useful in diagnosing AMI.

Puleo and Perryman also studied CK-MB isoforms following thrombolytic therapy. They found that the peak ratio MB_2/MB_1 was >3.8 in 18/20 patients with reperfusion of the infarct related artery compared to <3.8 in 17/19 patients in whom reperfusion did not occur. The time to peak ratio was of the order of 2 hours after treatment, so allowing identification of a group of patients who may benefit from intervention, for example, salvage PTCA or repeat thrombolysis (Puleo,1991).

Measurement of CK-MB isoforms would appear to have a potential application in both the diagnosis of AMI, and identifying patients who do not appear to have reperfused. The main disadvantage in their utilisation would appear to be that thay are measured by electrophoresis, which is usually a slow process. This may be overcome by using high voltage electrophoresis, with a reported assay time of 25 minutes (Puleo,1990).

1.4.6 CREATINE KINASE-MM BANDS

CK-MM has been found in more than one isoform in the serum of man (Morelli,1983). Initially, CK-MM_3 is released from myocardium, this is converted to CK-MM_2 then CK-$MM_1$1. A peak in the ratio of CK-MM_3 to CK-MM_1 is seen (Collinson,1988). Muller-Hansen describes this peak at 7 hours, and the ratio MM_3:MM_1 to return to normal within 27 hours. In the presence of a normal CK

value, a ratio MM_3:MM_1 >1 gives a sensitivity of 86%, but a lower specificity, for the diagnosis of AMI, and its use in determining who receives thrombolysis would appear to be limited by this number of false-positive results (Muller-Hansen,1989). Although this ratio does rise within 12 hours following AMI, the analysis is performed by electrophoresis, and so requires at least 2 hours separation time. Consequently, its applicability to routine clinical use remains doubtful.

In a similar study, Jaffe et al, using a nomenclature of CK-$MM_{A,B,C}$, instead of CK-$MM_{3,2,1,}$ showed that following AMI, the ratio of MM_A to MM_C in the first available plasma sample was increased at a time when total CK and CK-MB levels were not raised (Jaffe,1986). Although he commented that this showed CK isoform profiles to alter soon after AMI, the analytical process was complicated, requiring 6 hours of electrophoresis and 4 hours exposure to X-ray film.

1.4.7 MYOGLOBIN

The measurement of myoglobin following AMI was first described by Kagen et al in 1975 (Kagen,1975). Myoglobin is a low molecular weight protein (17,800 D), found in cardiac and skeletal muscle. It is released following tissue necrosis, and can be measured in serum and urine. It is present in low concentration in the serum of healthy individuals (6-85 μmol/l).

The main disadvantage in the use of myoglobin as a marker of AMI is that it is not cardio-specific. If there is concurrent skeletal muscle damage it has no diagnostic role to play, despite the observation that the ratio of myoglobin to carbonic anhydrase is elevated after AMI, but is unchanged following trauma to skeletal muscle because carbonic anhydrase is present in skeletal muscle but not in

cardiac muscle. It is recognised that this is too cumbersome a technique to be used clinically (Vaananen,1990).

Cairns et al compared the kinetics of myoglobin release in 21 patients with QAMI to those of creatine kinase. They found that myoglobin was detected earlier, peaked earlier and disappeared at a faster rate than CK (Cairns,1983). This finding was confirmed by Mair et al using an immunoturbidimetric assay system which gave a result from a serum sample within 1-2 minutes (Mair,1991a), and by Kilpatrick et al although they compared myoglobin to CK-MB (Kilpatrick,1993). For the first blood sample obtained after admission, myoglobin was more sensitive, specific and had greater positive and negative predictive values for AMI than CK activity measured in the same sample. Carpeggiani et al showed that myoglobin fared better than CK-MB, correctly identifying 80% of the patients with sustained ST segment and/or T wave changes that were finally assumed to represent myocardial necrosis (Carpeggiani,1989).

A latex agglutination method for detecting myoglobin concentration was assessed by Collinson et al. This gave a positive result for a level >100 μg/L, the test result being available within 3 minutes. This method had a sensitivity and specificity of diagnois of 69% and 93% respectively (Collinson,1988). Ohman et al used a rapid, non-laboratory based agglutination test for myoglobin and showed it to be a useful addition in the diagnosis of AMI in those patients without ST elevation (Ohman,1990b). Not all workers have found the rapid agglutination test reliable (Fitzgerald,1988).

1.4.7.1 EFFECT OF REPERFUSION ON SERUM MYOGLOBIN CONCENTRATION

Ishii et al compared serum myoglobin levels to CK-MB levels following thrombolysis (Ishii,1991). They found that in patients in whom reperfusion was demonstrated at angiography, the rate of appearance of myoglobin was initially greater than that of CK-MB, although both were similar at 60 minutes. However, from 15 to 60 minutes, myoglobin was a more reliable indicator of coronary artery reperfusion. A similar result was found by Ellis et al who showed that in patients with successful reperfusion, the time to peak myoglobin occurred earlier with a rise in concentration of >4.6 being seen in the first 2 hours after thrombolytic therapy in the majority of patients (Ellis,1988), whereas the converse applied to those patients who did not reperfuse. Miiyata et al found a cut-off level >2 at 60 minutes to be diagnostic of reperfusion (Miiyata,1994). Clemmensen et al showed that a peak myoglobin value occurring within 4 hours of the administration of thrombolytic therapy was highly accurate for detecting reperfusion (Clemmensen,1991). Abe et al compared the rate of rise after 1 hour of myoglobin (9.7 ± 9.5 hours), and CK (2.8 ± 1.6), and found them to be significantly different (Abe,S,1993). Dillon et al measured myoglobin at the time of admission and 60 minutes later and showed that if the rate of rise divided by the value at the time of commencement of therapy was greater than 0.234 ± 0.409 min^{-1} this was a reliable indicator for reperfusion to have occurred (Dillon,1991).

Katus analysed the times to peak value of myoglobin, CK-MB and CK following recanalisation of the infarct related artery with intra-coronary streptokinase (Katus,1988b). In those patients receiving thrombolytic therapy within

3.5 hours of the onset of symptoms, reperfusion could be predicted with a probability of 0.9-1.0. In those cases where reperfusion took place greater than 3.5 hours after the onset of symptoms, reperfusion could not be reliably predicted. In the "early" group, myoglobin values peaked at 5 hours, CK-MB at 11 hours and CK at 12 hours after the onset of symptoms. Zabel et al also found myoglobin to be the best predictor of reperfusion at 90 minutes, when compared to CK, CK-MB and troponin-T (Zabel,1993).

The measurement of myoglobin and CK concentrations before thrombolysis is given provides opportunity to determine which patients, if any, have undergone spontaneous reperfusion. Abe et al comment that if the Myoglobin/CK ratio >5.0 on admission then there is no requirement for thrombolysis (Abe,J,1993). This contentious view is not currently accepted as valid clinical practice and would require 100% sensitivity to ensure that no patients were withheld thrombolytic therapy incorrectly.

The combination of biochemical marker change and ECG monitoring is proposed by Baskin et al who argue that the individual sensitivity for predicting reperfusion is 62% for ST segment resolution and 75% for certain myoglobin concentration changes. The combination of these 2 parameters would be likely to increase this significantly (Baskin,1993). With regard to prognosis, assessing the size of infarction after reperfusion by calculating the sum concentration can be determined more quickly using myoglobin than CK, approximately 3 hours to 24 hours respectively (Yamashita,1993).

Myoglobin therefore appears to provide the earliest and most accurate biochemical prediction of infarct reperfusion and it would appear that measurement

of myoglobin concentrations can reduce the time following thrombolysis to further intervention (if available) to about 2-4 hours plus the time to perform the assay. A major drawback is that infarct reperfusion only seems to be reliably predicted in those patients that present early, but since this is the group that is most likely to have myocardium that can be salvaged then this may not be too much of a disadvantage (Stack,1983). It is also important to note the observation by Katus et al that not all patients are likely to show the same biochemical response to successful reperfusion, and equally, not all patients are likely to benefit from intervention in the immediate post-infarct period. The need for further studies to identify which patients benefit most from such management is apparent.

1.4.8 TROPONIN-T

Troponin-T is a contractile protein present in myocardium (Cummins,1990). It is one of the proteins of the contractile apparatus that are unique in their primary structure for cardiac muscle (Katus,1991a). Cardiac troponin T is a unique myocardial antigen, and can be readily differentiated from the skeletal muscle isoform (Editorial,1991). It has a molecular weight of 37 KDaltons (Potter,1974).

Troponin-T has been evaluated as a diagnostic marker for AMI. Katus et al found a sensitivity of AMI diagnosis of 100% (Katus,1991a). Specificity was less precise, 48 of 210 patients without AMI also had elevated levels. Of these 37 had unstable angina and 11 had atypical chest pain. In those patients with unstable angina, the raised concentrations of troponin T were highly specific for severe coronary artery narrowing. Bakker et al found similar results with the sensitivity of diagnosis highest for troponin-T, but specificity lowest, although they did choose a

diagnostic cut-off of 0.1ng/ml (Bakker,1994). Increasing the cut-off to >0.5ng/ml increased specificity, without compromising sensitivity, although this study only included patients with relatively late presentation (Mair,1991c). Combining the measurement of troponin-T with CK-MB in patients presenting early improved the specificity to 92% (Katus, 1991a).

A number of workers are investigating the diagnostic potential of troponin-T in patients with unstable angina, and the presence of so-called micro-infarction (Ravkilde,1993), as well as using very small rises in troponin-T (just below the AMI diagnostic cut-off) to indicate prognosis. Those patients with very small increases appear to have a worse prognosis (Bakker,1994). In patients with co-existent trauma, especially skeletal muscle damage, troponin-T was considerably more accurate than CK-MB at diagnosing AMI, because of its cardio-specificity.

The release kinetics of troponin-T differ markedly from those of CK-MB. It appears slightly earlier, and peaks on day 1 to a value up to 40 times its "normal" detectable concentration. It also has a second peak at day 4 and can be measured up to 12 days after the index AMI. A diagnostic window of 10.5-140 hours from the onset of symptoms is apparent, casting doubt upon any further role of AST or LDH in the late or retrospective diagnosis of AMI. Its release kinetics suggest it to be present both freely in the cytoplasm, and to be structurally bound. The process of degradation of myofibrillar proteins is time consuming and barely affected by blood flow to the infarct zone (Sahida,1984). The primary release of troponin T would seem to be due to leakage from the cytoplasm of ischaemic but partly viable myocytes, this would explain the small rise seen in patients with unstable angina (Katus,1991a). The later peak, usually around day 4 therefore probably reflects

damage to the subcellular contractile apparatus (Piper,1984).

1.4.8.1 EFFECT OF REPERFUSION ON SERUM TROPONIN-T CONCENTRATIONS

The appearance of troponin-T in serum on day 1 following AMI is dependant upon reperfusion and duration of ischaemia. In patients with early reperfusion a troponin-T peak is found which is absent in patients with reperfusion occurring more than 5.5 hours after the onset of symptoms, and in patients in whom reperfusion does not occur. Katus measured troponin-T levels at 14, 32 hours and peak concentration after the onset of symptoms and found the ratios: peak/32 hours >1.1 and 14/32 hours >1.0 to be similar with regard to sensitivty (90%), specificity (100%), positive predictive value (100%) and negative predictive value (65%) in the differentiation of patients with and without successful reperfusion (Katus,1991b). It follows that the level measured at 14 hours will suffice as the "peak" value in the majority of patients, and more importantly, can be used as such for practical purposes. This was confirmed by Rempiss et al who reported a probability of reperfusion of $>95\%$ if the peak concentration:38 hour concentration ratio >1.42, or the peak:14 hour concentration ratio >1.09, although this technique clearly requires knowledge of the peak concentration, this would appear to be approximately 12-15 hours after reperfusion (Remppis,1994). Utilising an actual rise rather a rate of rise in concentration, Abe et al found an increase in troponin-T concentration of 0.5ng/ml to be a highly sensitive indicator of reperfusion (Abe,S,1994).

Other isoforms of troponin have been studied in relation to AMI diagnosis.

Cummins et al raised antibodies to troponin-I and using radiolabelled scintigraphy in a dog model showed uptake of labelled antibodies to be up to 24 times greater in necrotic than in normal myocardium (Cummins,1990). Studies in man show it to be both highly sensitive (Adams,1993) and highly specific for myocardial injury (Ordonez-Llanos,1994). At present, there is far less information available for troponin-I than troponin-T and the latter is presently better established for clinical purposes.

In summary, troponin-T is not normally detectable in serum. It is a cardiospecific antigen and because it is mostly located in the sub-cellular compartment, it is released over a number of days rather than hours. These characteristics provide it with the ability to become a useful addition to the various markers currently employed in the diagnosis of AMI, both acutely and retrospectively, as well as having a potential use in the assessment of infarct size.

1.4.9 CREATINE

Serum creatine concentrations are usually low (DeLanghe,1988). They increase slightly with age, and are a little higher in females. The normal range is of the order of 20-70 μmol/l. Reference values are lower in vegetarian subjects (DeLanghe,1989), whereas prolonged bed rest results in increased serum levels (Zorbas,1990).

Creatine phosphate is found intra-cellularly. It functions as a phosphate donor, being a store of high energy intra-cellular phosphate. The position of equilibrium in the reaction creatine ⇌ creatine phosphate favours creatine phosphate formation. Consequently intra-cellular creatine concentration is usually low

(approximately 9% of total intra-cellular creatine); intra-cellular creatine phosphate stores are usually high (approximately 55% of total intra-cellular creatine). About 36% of total intra-cellular creatine exists bound to unknown intra-cellular components (Savabi,1988).

In conditions of acidosis or hypoxia this situation changes (Sugden,1991; Carter,1990). For example, following myocardial infarction, intracellular adenosine triphosphate (ATP) stores rapidly become depleted, intracellular pH falls and as a consequence, intracellular free creatine rises. Disruption of the cell membrane allows creatine to enter the circulation; this is facilitated by its low molecular weight (m.w. 131).

The renal threshold for creatine excretion is roughly similar in all adults (of the order of 68 ± 12 μmol/l), which is slightly higher than the upper limit of normal serum concentration. If serum creatine concentration becomes elevated, urine creatine concentration increases. It has been proposed that a concentrating effect occurs, with urinary concentrations being higher than plasma concentrations (DeLanghe,1988).

DeLanghe investigated creatine levels in serum and urine in 22 patients with QAMI. He found that a detectable rise in serum creatine occurred within 2-4 hours from the onset of symptoms, but that because of a concentrating effect, levels in urine were far more discriminatory. He proposed that creatine concentrations in serum and urine could be used in the early diagnosis of AMI (DeLanghe,1988). However, in this study, more than 50% of the patients required urinary catheterisation, reflecting a group of patients sustaining large infarcts. Although he showed that interfering effects from other tissues could be discounted, these results

clearly require validation with a larger, more representative sample of patients being admitted to a Coronary Care Unit.

1.4.10 MYOSIN

Cardiac myosin light chains are structurally and immunologically specific for cardiac tissue (Sarkar,1971). They are not normally detectable in serum (Katus,1984). Katus measured the peak and cumulative myosin light chain (MLC) concentration following AMI (Katus,1988a). Whereas cytosolic proteins such as CK are dependent upon early reperfusion for their appearance in plasma, MLC are released gradually over a 14 day period. He concluded that they may give rise to useful prognostic information about infarct size. MLC are measured by a competitive binding RIA (Hoberg,1987a), which also makes them unlikely to be useful for an acute diagnostic purpose.

Hoberg et al compared a number of markers in patients with unstable angina and found that MLC levels were only increased in patients with a coronary artery stenosis of at least 70% (Hoberg,1987b). Patients with 3 vessel disease were also more likely to have raised levels than those with 1 or 2 vessel disease. They propose that these small and not uniform rises indicate small areas of necrosis, so called microinfarcts. Such lesions are noted in patients with unstable angina who have died with no clinical evidence of infarction (Robbins,1963). If this is indeed the case, then of the various markers evaluated, MLC appear to be one of the most sensitive indicators of AMI.

Nicol et al used a newly developed radio-immunoassay for ventricular myosin light chain 1 (VLC1) and showed that times to appearance (4.7 ± 0.68 h)

and peak value (54.4 $\pm$ 5.8 h) were similar to those of CK-MB, although VLC1 levels were not influenced by reperfusion therapy (Nicol,1991).

1.4.11 OTHER BIOCHEMICAL MARKERS

A number of other naturally occurring substances have been investigated with regard to diagnosing AMI.

Protein S100 belongs to the family of proteins that includes calmodulin, troponin-C and the light chain of myosin. Serum-$100a_0$ protein is the α-α isomer and is present in high concentration in myocardium. Usui et al measured serum-$100a_0$ levels by enzyme immunoassay and found them to peak 8 hours after admission and to gradually return to normal. There was satisfactory discrimination between patients with AMI and patients with angina pectoris (Usui,1990).

Endothelin is a vasoactive peptide recently isolated from the supernatant of endothelial cells in culture. It is a potent constrictor of isolated coronary arteries from animals and humans. Stewart et al measured endothelin-1 levels in patients with AMI. They found that plasma levels increased rapidly after AMI, reaching a peak 6 hours after the onset of pain, and returned to normal by 24 hours, except in patients with haemodynamic disturbance whose levels were generally higher and remained elevated for up to 72 hours (Stewart,1991). There was no correlation of peak CK with endothelin in either of the 2 groups of patients, suggesting that the stimulus for enothelin release is not myocardial necrosis, but more likely to be a manifestation of endothelial disruption, myocardial ischaemia or depressed left ventricular function.

A rapid increase in renal permeability to plasma proteins after trauma, burns,

surgery or ischaemia is recognised, this appears to be proportional to the severity of the insult (Fleck,1985). Gosling et al measured urinary albumin concentration following AMI and found a significantly higher urinary albumin:creatinine ratio in the AMI compared to non-AMI group (Gosling,1991). Urinary IgG:creatinine ratios also increased, suggesting the proteinuria to be at least partly secondary to an increase in glomerular permeability. This would be compatible with the suggestion that myocardial ischaemia/necrosis results in a systemic increase in vascular permeability as part of the acute inflammatory process (Suval,1987). A combination of albumin:creatinine and IgG:creatinine ratios gave a sensitivity and specificity of AMI diagnosis of 82% and 96% respectively, and the speed with which these can now be performed by immunoturbidimetry may allow urine to be incorporated into the diagnostic process. The main difficulty related to obtaining a sample of urine, in this study the range of times from onset of symptoms to passing the first urine sample was 2-24 hours (mean 10.9) in the AMI group, compared to 1.5-24 hours (mean 11.1) in the non-AMI group.

Bhatnagar et al measured blood taurine levels in patients with presumed cardiac chest pain and found levels to be higher in patients with AMI or unstable angina than controls (Bhatnagar,1990). Taurine levels were higher in patients with AMI than unstable angina but they commented that, at present, they did not feel that the test could reliably discriminate between these 2 acute coronary syndromes.

2,3-butanediol is a product of intermediary metabolism. Heer et al measured it in blood samples taken after AMI and showed levels to be higher in comparison to a control population, but within the AMI group only 64% had an elevated level considered to be diagnostic (Heer,1990). The release characteristics were completely

different for 2,3-butanediol in comparison to CK; whereas levels of the former had returned to normal within 24 hours, levels of CK-MB continued to be elevated for at least 48 hours or so.

Peripheral atrial natriuretic factor (ANF) levels increase following AMI (Gutierrez-Marcos,1991). Ngo et al suggest that this can be used to aid in the diagnosis of AMI, but ANF levels are affected by giving thrombolytic therapy, presumably secondary to the haemodynamic effects of intravenous streptokinase. Ray et al argue that because of this, ANF cannot be used as a reliable indicator of AMI (Ray,1990), this view is shared by Tan et al who observed no relationship between ANF values and the clinical course following AMI (Tan,1989).

1.4.12 SUMMARY

The criteria for a suitable test of acute myocardial infarction are:
It must be available 24 hours a day, be rapidly interpretable and be diagnostic very early after the onset of symptoms. In the post thrombolytic era it is an advantage if it can make a contribution towards assessing coronary artery reperfusion.

It is clear that of the markers outlined above, some meet these criteria more so than others, but it is also apparent that that they all have disadvantages as well as advantages. The need to consider and evaluate these and other markers in the roles of diagnosis and differentiation of AMI, as well as assessing the effects of thrombolysis is real, especially in light of continuing developments in the management of the various subsets of patients identified post-AMI.

CHAPTER 2: AIMS OF THE INVESTIGATION

AIMS OF THE INVESTIGATION

1) To compare serum and urinary creatine concentrations to recognised biochemical markers in the early diagnosis of acute myocardial infarction.

2) To evaluate the role of serum and urinary creatine concentrations and other biochemical markers in the differentiation of acute Q-wave and non Q-wave myocardial infarction.

3) To evaluate the role of serum creatine and other biochemical markers in conjunction with recognised electrocardiographic criteria, in the non-invasive diagnosis of coronary artery reperfusion following intra-venous thrombolytic therapy.

CHAPTER 3: PATIENTS AND METHODS

CHAPTER 3 - PATIENTS AND METHODS **PAGE**

PATIENTS AND METHODS

3.1 PATIENTS

Patients admitted to the Coronary Care Unit at Pinderfields General Hospital with a differential diagnosis including acute myocardial infarction were studied.

Informed consent was obtained prior to study entry.

The study protocol was approved by the local Research Ethics Committee prior to commencing the study.

3.2 METHODS

3.2.1 12-LEAD ELECTROCARDIOGRAPHY

12-lead electrocardiograms were recorded by a Hewlett-Packard "Pagewriter" electrocardiograph, with the patient in the supine position. Standard electrode positions were used, except for a modified precordial V5 position, once the Holter monitor had been hooked-up to the patient. Disposable "Medicotest" electrodes were used for each patient.

3.2.2 AMBULATORY ST SEGMENT MONITORING

3.2.2.1 INTRODUCTION

24 hour ambulatory monitoring of heart rhythm and ST segments was performed in all patients. The usefulness of these two aspects of electrocardiography to monitor myocardial infarction and ischaemia, and the effects of thrombolysis in the immediate post-infarct period is currently of great interest.

3.2.2.2 TECHNICAL CONSIDERATIONS

The electrocardiogram is composed of a number of signals of varying frequency, the lowest frequencies being in the ST segment, the highest frequencies are found in the QRS complex. The American Heart Association has issued which it regards should be met by recorders used for ambulatory monitoring of the electrocardiogram (Report,1975; Knoebel,1989). There is a consensus that the range of frequencies monitored is at least 0.5-60 Hz (Bragg-Remschel,1982; Tayler,1988; Silber,1990), with appropriate low frequency recording characteristics (<2dB at 0.05Hz with minimal phase shift (Rotman,1988)), since an inadequate low frequency response results in artificial ST segment/T wave changes (Hinkle,1967). The sampling frequency should be twice the upper frequency cut-off, namely 120 Hz (Reynolds,1990). A linear phase response should be present within the band width 0.5-60 Hz (Tayler,1988).

Continuous tape recording has a number of inherent technical problems. It is important to keep the recording head clean and aligned with the tape, and it should be regularly de-magnetised (Krucoff,1989). Tape speed should be uniform so as to eliminate tape motion artefact (Reynolds,1990). This is especially important when heart rate variability analysis may also be required. If the tape is repeatedly analysed. information can be lost, especially if the x60 speed is used for analysis (Rotman,1988).

3.2.2.3 ELECTRODE POSITIONS

The number of channels which can be recorded at any one time is limited to the type of recorder. Some authors claim that recording from less than 12 leads is

not ideal and should therefore be discouraged (Saran,1990); whilst some advocate using recorders with 3 channels (so allowing an inferior, anterior and lateral lead position to be monitored) (Krucoff,1989). However, most commercially available recorders have 2 channels on which to record, and when used appropriately, this number is regarded as sufficient (Rotman,1988).

Within the limitations imposed by recording from only 2 channels it is important to ensure that the electrode posistions selected will detect all ECG changes that may occur. It is generally agreed that for antero-lateral myocardial ischaemia, bipolar lead CM5 (reference electrode manubrium sterni, active electrode V5), is most effective at detecting ST segment changes in this territory (Quyyumi,1986; Huey,1987). Silber et al reported a 96% sensitivity for this lead configuration for all anterior ST segment changes (Silber,1990), Quyyumi et al reported a sensitivity of 93% (Quyyumi,1986). Using this lead position, Hoberg et al detected ST segment changes in all 26 patients undergoing angioplasty to any coronary artery (Hoberg,1987).

However, Jespersen and Rasmussen showed that this lead position failed to detect ST segment changes occurring inferiorly in 1 of 30 angioplasty cases (Jespersen,1988), and Quyyumi et al found similar results in 1/3 of angioplasties to the right coronary artery (Quyyumi,1986).

In light of this, a number of modifications to standard lead positions have been tried, to try to improve the sensitivity and specificity of ST segment changes detection associated with ischaemia of the inferior myocardium. Thus, a modified orthogonal y lead (-ve electrode at the superior angle of the scapula, +ve electrode above the left iliac crest) and a modified lead III have been shown to be more

effective than lead CM5 at detecting inferior ST segment changes (Huey,1987; Hombach,1990). Osterhues affirms that a Nehb-D like lead position (indifferent electrode placed in the right first intercostal space, midclavicularly and the exploring electrode beneath the scapula) significantly increases detection of inferior ST segment changes (Osterhues,1994). It has been suggested therefore that for a 2 channel system a combination of lead CM5 and one of these leads be used (Rotman,1988; Hombach,1990).

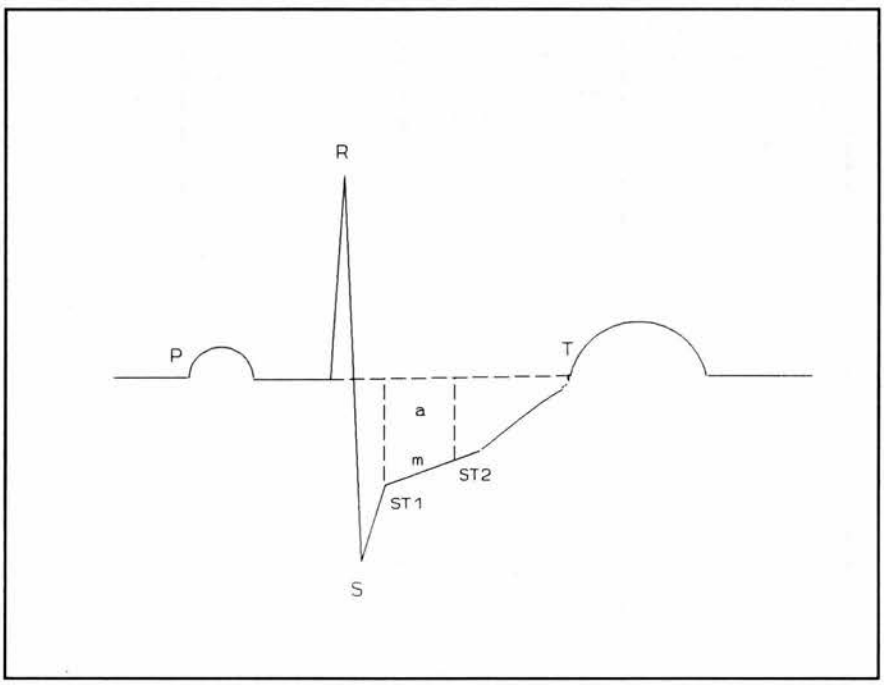
In order to minimise artefactual interference of the ECG signal it is essential that attachment of the electrodes and cables to the patient be given detailed attention. The skin-electrode interface is very important, being the first step in obtaining a good quality recording (Balasubramanian,1980; Krucoff,1989).

3.2.2.4 ST SEGMENT ANALYSIS

When analysing the ST segment either by visual or automated means, a single "normal" ECG waveform acts as a template, as in the Neilson arrhythmia detection algorithm (Reynolds,1990). False positive events are minimised by the use of intelligent rate adaptive thresholds. This approach of updating a stored normal QRS provides a means of compensating for changes in morphology arising from the following factors that influence ST segment interpretation:

Left and right ventricular hypertrophy, electrolyte imbalance, conduction disturbances, autonomic change, certain drugs, hypertension, mitral valve prolapse, posture and hyperventilation (Huey,1987; Rotman,1988).

A number of points on the ECG waveform act as reference points for ST segment analysis. These are as follows:



KEY

P = start of P wave

R = R wave

S = S wave

T = start of T wave

ST1 = J point (end of QRS complex)

ST2 = J point + t (where t = fixed interval, usually 80 milliseconds)

Isoelectric line = horizontal line through preceding TP points

m = gradient of slope ST1 to ST2

a = area bounded by ST1 and ST2 verticals, slope ST1 to ST2, and the isoelectric line.

The isoelectric line is taken as a horizontal between the preceding T and P points. The fiducial point is measured at an interval before the R wave, for example 56, 64 or 72 milliseconds (ms) (Silber,1990). For the measurement of both ST segment elevation and depression, the J point (ST1) is decided upon by manual technique and acts as the reference for all other measurements (Gallino,1984; Rotman,1988).

Measurement of ST segment elevation is considered valid at the J point, (Saran,1990; Hogg,1988), although Huey et al measured it at J+40ms (Huey,1987). A given value for ST segment elevation at any one time is calculated by taking the mean of three consecutive complexes (Hogg,1988; Saran,1990).

There is some debate as to how to measure ST segment depression. Measurement of ST2 is usually made 80 ms after the J point by the majority of investigators (Quyyumi,1986; Huey,1987). However, ST segment changes are also regarded as valid if measured at 60 ms after the J point (Krucoff,1989), or 96, 104 or 112 ms after the R wave (Silber,1990).

True ST segment depression is interpreted as at least 1mm depression at both ST1 and ST2, that is, horizontal or downsloping depression (Ribisl,1993). If depression of only one of these points is used, this overestimates the incidence of ischaemic change by 20% (Rotman,1988).

A gradient of the slope joining ST1 and ST2 of greater than 1 mV/s is regarded as significant, although this has only 50% of the sensitivity of ST segment depression at detecting ischaemia. An area under the isoelectric line that is bounded by the ST segment of greater than 10mVs (465) is regarded as having only 10% of the sensitivity of ST segment depression for this purpose

(Rotman,1988).

If a change in the ST segment is to be regarded as significant, it must be present for a certain length of time. The exact duration is not clear, but a range of 30 to 60 seconds has been suggested (Silber,1990). There must be at least 1mm amplitude (Balasubramanian,1980; Quyyumi,1986; Krucoff,1989) and a return to baseline values for at least 2 minutes before a subsequent episode can be regarded as distinct from its predecessor (Gallino,1984). As for ST segment elevation measurement, the mean of 3 consecutive PQRST complexes at any given time during the recording is regarded as the actual value at that particular moment in time (Saran,1990).

Due to the large amount of data acquired by any one 24 hour recording, the automated analysis of tapes by a computer with an algorithm for detecting the above changes is used. This has been shown to be at least as accurate, and in some cases, more accurate, than visual inspection of the tapes (Gallino,1984; Rotman,1988).

3.2.3 ST SEGMENT MONITORING PROCEDURES USED

3.2.3.1 AMBULATORY RECORDER

Reynolds Medical "Tracker" recorders which allow 2 separate channels and a time signal to be recorded continuously, were used. Reynolds "Tracker" recorders meet the technical requirements outlined above, and are deemed suitable for high quality ambulatory ECG monitoring. The Tracker uses a direct recording system for the detection of arrhythmias, and ST segment monitoring is performed following all QRS complexes of less than 110ms duration (Tayler,1988).

In accordance with the manufacturer's instructions, a new Duracell 9V

battery and TDK D90 tape cassette were used for each recording. A new tape was used for each patient, to allow a permanent record to be kept.

At the start of each recording the tape was calibrated (1mm = 1mV). Although re-calibration during recording has been suggested by some authors (Rotman,1988), this was not performed. Where possible, prior to commencing recording the patient was placed in a number of different positions (supine, left lateral, right lateral and sitting), these were correlated with the time on the recorder clock. This allowed for any baseline variation or ST segment changes associated with patient movement to be identified, and excluded from subsequent analysis.

3.2.3.2 ELECTRODE POSITIONS

A combination of leads CM5 and modified lead III were used for all patients, with electrodes being placed in the right infraclavicular, left infraclavicular, left 5th intercostal space in the mid-clavicular line and right 5th intercostal space midway between the sternum and mid-clavicular line. These electrode positions were chose because all electrodes could be attached to the front of the patient's chest. Since all patients were confined to bed rest, it was felt that electrodes attached to the back of the chest would be prone to increased interference artefact and even become dislodged.

Prior to attaching the skin electrodes, satisfactory position was assessed by using suction electrodes and a monitor cable (connected to the "Tracker") attached to a bedside ECG monitor. This provided confirmation that the proposed electrode positions gave an ECG signal that would be suitable for subsequent analysis. Once the electrode positions were verified, the following skin preparation was performed:

The skin was shaved (if necessary), cleansed with alcohol, and lightly abraded with a gauze swab. The electrode position was marked with a water resistant pen to allow repeat electrode placement if necessary. Skin impedance was considered satisfactory if less than 5kOhms. Pre-gelled electrodes (3M Health Care "Red Dot" diaphoretic electrodes) were used. Electrodes were sited over a bony prominence to reduce muscle artefact; movement of free cables and the recorder-cable junction was kept to a minimum by taping the leads to the patients chest.

3.2.3.3 ANALYSIS OF TAPE RECORDINGS

Tape analysis was performed by a Reynolds "Professional" analyser. Analysis by the "Professional" system employs the Neilson arrhythmia detection algorithm. Tapes were analysed up to a maximum speed of 24 hours of recording in 10 minutes. This provided heart rate and ST segment analysis on both channels with time channel calibration.

Within each individual tape analysis, a number of options were available. An ST overview was provided which allowed the complete 24 hour period to be visually assessed for regions of interest. Once these regions had been identified that portion of the recording could be recalled and subjected to further, more detailed, analysis.

3.2.4 BLOOD SAMPLES

Twenty millilitre (ml) blood samples were taken into plain tubes and allowed to stand, for no longer than 4 hours. The tubes were centrifuged at 500g for 10 minutes, the serum separated and decanted into sterile 5ml plastic containers in at least 2 aliquots, and frozen immediately. Serum samples were stored at -70°C. All samples were given a unique identification code and labelled with permanent, water resistant marker pen.

For analysis to be performed, serum samples were thawed slowly to room temperature, mixed thoroughly and centrifuged at 1000g for 5 minutes. Samples were then transferred to individual cups compatible with the various auto-analysers used for the assays described below.

3.2.5 URINE SAMPLES

Urine samples were collected in as sterile a manner as possible (as for a mid stream specimen of urine). They were placed immediately into sterile 20ml containers and refrigerated. Within no more than 4 hours an individual sample was divided into 2 x 5ml aliquots, placed into sterile plastic containers and frozen. Samples were stored at -70°C. All samples were given a unique identification code and labelled with permanent, water resistant marker pen.

For analysis to be performed, urine samples were thawed slowly to room temperature, mixed thoroughly and centrifuged at 1000g for 5 minutes. Samples were then transferred to individual cups compatible with the various auto-analysers used for the assays described below.

3.2.6 BIOCHEMICAL ASSAYS

3.2.6.1 CREATINE KINASE-MB MASS DETERMINATION

3.2.6.1.1 PRINCIPLES UNDERLYING ASSAY PROCEDURE

Measurement of serum CK-MB concentrations was performed by a commercially available assay, ICON QSR CK-MB (Hybritech, Nottingham, UK). The assay detects CK-MB in serum by using two different monoclonal antibodies that react with two different regions of the CK-MB molecule. One monoclonal antibody binds to the B subunit, the other binds to the M subunit.

The antibody to CK-B is contained in the test zones of the ICON QSR filter membrane and captures and immobilises the CK-MB molecules contained in the test sample. The antibody to CK-M is chemically linked to the enzyme alkaline phosphatase, and binds to the CK-MB molecules immobilised on the test membrane. The immobilised CK-MB molecules are thus "sandwiched" between the solid phase and enzyme linked monoclonal antibodies.

3.2.6.1.2 ASSAY PROCEDURE

All assays were performed on fresh serum from blood samples which had been taken no greater than 4 hours prior to the assay. All reagents and test materials were brought to room temperature prior to the start of the assay. Good attention to detail was employed to ensure that there was accurate pipetting technique, no contamination of solutions,and the avoidance of air bubble formation in the specimen cup or when adding solutions to the test membrane. Reagents from each individual kit were only used for that particular kit.

Initially, 75μl of specimen pre-treatment solution was pipetted into a

specimen cup, followed by 300μl of serum. These were mixed and allowed to incubate for one minute. (This stage is designed to remove any heterophilic anti-mouse antibody contained in the specimen sample that would react with the anti-CK-M and anti-CK-B monoclonal antibodies). After incubation, 300 μl of this treated sample was pipetted onto the test membrane and allowed to drain completely. The solution containing anti-CK-M antibody linked to alkaline phosphatase was added; 3 drops in rapid succession to the centre of the test membrane. After a further 3 minutes, a wash solution was applied, allowed to drain, and the repeated. A solution containing a substrate for alkaline phosphatase was added; 3 drops in quick succession to the test membrane. The development of a colour change during this stage was dependent upon the presence of alkaline phosphatase, which was related directly to the binding of anti-CK-M to the test membrane. After a 3 minute interval, a wash solution was added to quench the reaction, and allowed to drain.

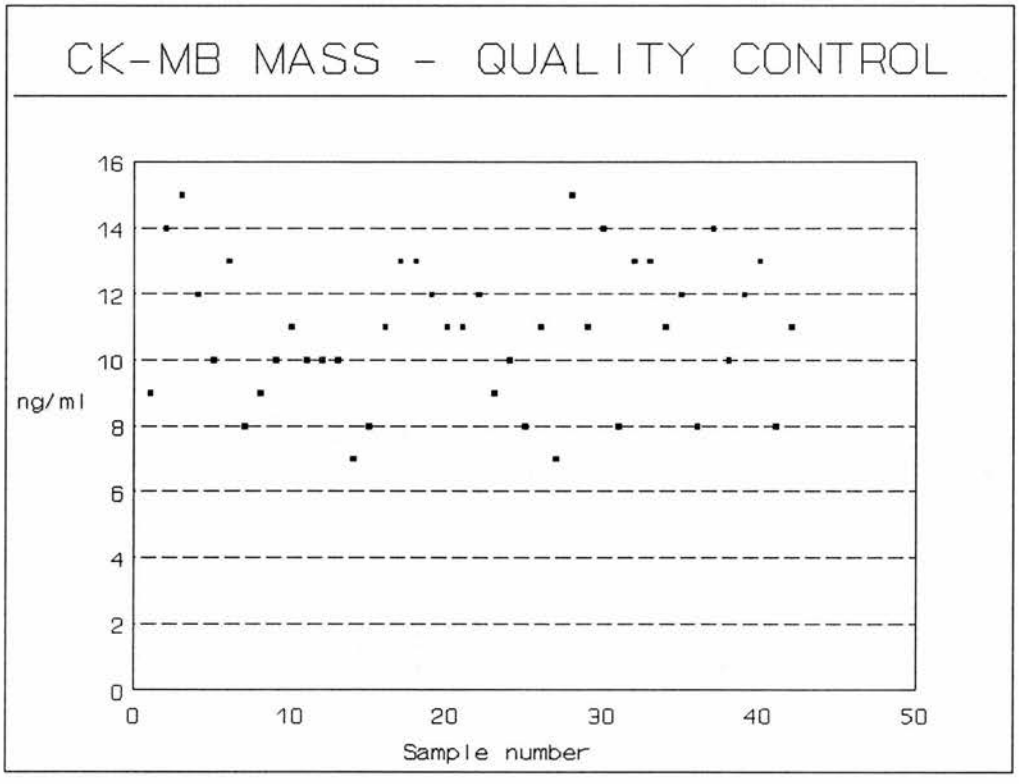
A dedicated ICON reader was required to read the test cylinder. This was standardised prior to each measurement. The ICON reader compared colour development at the test zones with that of the calibrator zones. The test cylinder was inserted into the reader and the reflectance at 585nm was read; the response was directly proportional to the amount of enzyme bound to the membrane, which itself was directly prortional to the amount of CK-MB present in the specimen. A value of mass concentration of CK-MB was given with a range of 2-50 ng/ml. Each test cylinder had 4 zones, 2 duplicate test zones, 1 high and 1 low calibrator zone. If the reflectance readings differed widely at the two test zones the assay was declared invalid and repeated.

3.2.6.1.3 QUALITY CONTROL

Positive and negative control specimens were provided by the manufacturer to allow comparison of assay performance during the course of the study.

Inter-assay variability

A positive control (range 5-15ng/ml) measurement was made each time an assay run was performed. The figure below shows the results of this, indicating the variation in measurement of CK-MB mass between assays.



Intra-assay variability

Randomly chosen samples were subjected to multiple assays, within a particular assay run. A total of 36 samples on 36 days were assessed, each sample being assayed 3 times. The range of CK-MB mass measurements was 1-90 ng/ml, the variation in measurement was $\pm$ 6.4%.

3.2.6.2 CREATINE KINASE-MB ACTIVITY ASSAY

The Kodak Ektachem slide method was used for this assay.

3.2.6.2.1 RATE METHODOLOGY

The Kodak Ektachem method employs a change in reflectance density of a variety of colourimetric indicators to calculate the initial concentration of a particular analyte in the test sample. For example, the concentration of CK-MB in the original sample is calculated from the rate of change of chromophore production during a 5 minute incubation period, the reflection density is read at 670nm and compared to a standard curve previously established during calibration of each batch of assays performed.

The Kodak Ektachem method mostly uses multiple-point rate tests, a total of 54 reflectance measurements being taken at the appropriate wavelength setting every 5-7 seconds. The reflection density is calculated from the measured reflectance. The change can either be a positive one, e.g. colour increasing, or a negative one, e.g. a fading of colour.

An algorithm is used to eliminate aberrant results (a maximum of one excessively high or low result being allowed for each sample), and the maximum rate of change in reflection density is calculated from a linear region of the graph chosen between early measurements (taken prior to the reactions reaching a steady state), and later readings beyond defined tolerances of slope and rate.

A calibration curve is formed from readings taken from a number of samples of known concentration of the substance being measured. When a reading for a particular sample is decided upon, this is compared to the calibration curve and a concentration of the analyte in the test sample calculated.

All subsequent analyses described that use the Kodak Ektachem analyser work on this underlying principle.

3.2.6.2.2 ASSAY PROCEDURE

The slide contained a dry, multi-layered analytical element coated on a polyester film support. The assay used anti-human CK-M antibody to inhibit CK-M subunit activity. The remaining CK-B activity, (which is presumed to be proportional to 50% of the CK-MB activity) was assayed in the folllowing way: An 11 μl drop of patient sample was added to the slide, and evenly distributed by the isotropically porous, reflective spreading layer. The following reactions then took place:

creatine phosphate + adenosine diphosphate	(creatine kinase) →	creatine + adenosine triphosphate
adenosine triphosphate + glycerol	→	L-α-glycerophosphate + adenosine diphosphate
L-α-glycerophosphate + O_2	→	dihydroxyacetone phosphate + H_2O_2
H_2O_2 + dye precursor	→	dye + 2 H_2O_2

The main disadvantage of this method was the assumption that CK-BB levels in peripheral blood were sufficiently low that they did not interfere with the assay.

3.2.6.3 SERUM AND URINARY CREATINE ASSAY

3.2.6.3.1 ASSAY PROCEDURE

A commercially available kit (Boehringer Mannheim Creatinine PAP) that is normally used to measure creatinine concentrations was modified to allow measurement of creatine in serum and urine samples. The method has been described previously (Beyer,1993). By omitting the first step of this assay, creatine is measured in the following way by an automatic analyser and uses an enzymatic colorimetric method:

creatine + H_2O → sarcosine + urea

sarcosine + O_2 + H_2O → glycine + formaldehyde + hydrogen peroxide

hydrogen peroxide +
2,4,6-tribromo-3-hydroxy benzoic acid + → quinone-imine dye +
4-aminophenazone quinone-imine dye H_2O +
→ coloured dye hydrogen bromide

It is assumed that the rate of formation of quinone-imine dye is proportional to the initial concentration of creatine in the test sample. Solutions of known creatine concentrations are used to calibrate the analyser. The absorbance of the final solution is read in a spectrophotometer at 510nm; this initial reading is denoted as A_1. The absorbance is then read 15 minutes later, this value is denoted as A_2. The concentration of creatine in the initial sample is calculated by subtracting A_1 from A_2, and comparing this absorbance value to the creatine concentration corresponding to that from the calibration curve.

Interfering substances

The only substance shown to interfere significantly with this assay is bilirubin, and even then only at a concentration of greater than 50mg/l; this results in artificially low values of creatine being derived.

Because the assay employs colorimetry, there is a possibility that turbid samples may give rise to artificially high levels. For this reason, lipaemic samples were not included in this analysis.

3.2.6.4 SERUM AND URINARY CREATININE ASSAY

A similar method was used to measure serum and urine creatinine concentrations, except that the first step of the assay (Boehringer Mannheim Creatinine PAP) was included. This step involves the hydrolysis of creatinine to creatine by creatininase, the creatine generated by this step is then subjected to the same process as described above.

Within the system of coupled enzymatic reactions used in this assay, the creatininase step is highly specific with regard to its substrate. To confirm the selectivity of the assay for creatine or creatinine (by performing or omitting the first step of the assay procedure), "control" samples of known concentrations of creatine and creatinine were made up by an independent operator. These were included in the test runs, the concentrations of these "control" samples were not revealed to the investigators until all the results had been obtained.

3.2.6.4.1 SAMPLE STABILITY

Fuller and Elia (Fuller,1988) studied creatinine to creatine conversion and found it to be an enzyme independent process. The position of equilibrium was in favour of creatine formation at pH 5.0, and in favour of creatinine formation at pH 3.7. However, 24 hours was required for 5% conversion in either direction, and this was maximal at physiological temperatures. At 4°C, conversion was negligible, and if samples were frozen, no conversion was detectable. In this study, all urine samples were stored at 4°C for a maximum of 4 hours before being frozen, and during analysis were kept at room temperature for only a few hours.

3.2.6.5 MYOGLOBIN ASSAY

3.2.6.5.1 PRINCIPLES UNDERLYING ASSAY PROCEDURE

Serum myoglobin concentrations were analysed using either the Behring "Turbitimer" or "Nephelometer" analyser. These are quantitative assay systems that work on an identical immunochemical method. Polystyrene particles coated with anti-human myoglobin antibody agglutinate in the presence of myolobin in the serum sample. This agglutination results in an increase in turbidity which is measured photometrically. Quantitative determination of the serum myoglobin concentration is achieved by measuring the maximum velocity of agglutinate formation (V_{max}), and the time required to reach V_{max}. The temperature of the reaction is measured automatically from the temperature of the cuvette holder and the temperature of the reagent vial. Myoglobin concentration is calculated by comparing the parameters described above with a calibration field. A separate reference curve is calculated for different batch of reagent, prior to using the analyser.

3.2.6.5.2 ASSAY PROCEDURE - TURBITIMER

The reagent was provided as a lyophilisate which was resuspended just prior to use in a buffer solution. All reagent and buffer was stored as recommended by the manufacturer and used prior to the expiration date. The reagent was warmed to room temperature prior to use.

Initially, 50μl of undiluted serum sample was pipetted into a plastic cuvette. The cuvette was placed into the chamber provided in the Turbitimer. Then, 500μl of reagent was added to the serum sample to initiate the reaction. The mean reaction time was 80 seconds. The myoglobin concentration of the serum sample was determined automatically and printed in units of μg/l.

Inter-assay variation was determined by measuring apolipoprotein control serum CHD at various times during each run of assays. Individual serum samples were also remeasured during the same, and different batches of reagent to determine intra- and inter-assay variability.

3.2.6.5.3 ASSAY PROCEDURE - NEPHELOMETER

All reagents and solutions were warmed to room temperature prior to use, stored in accordance with the manufacturer's instructions and used prior to the expiry date. Serum samples are placed into cups and positioned on the analyser. All steps in the assay protocol are performed automatically by the Nephelometer.

Patient samples are automatically diluted 1:20 with N Diluent.

Then, 80μl of diluted sample is added to 20μl of N myoglobin supplementary reagent working solution and 75μl N reaction buffer and pipetted into a cuvette.

Then, 75μl of N myoglobin reagent and 75μl of N reaction buffer is added to the

cuvette.

The reaction time is 12 minutes and the result is automatically determined and printed in units of μg/l. The measuring range is 25-400μg/l. If the initial assay is greater than the upper range limit the analyser repeats the above steps, but with the sample diluted to 1:100. For accuracy and precision control, N myoglobin control is assayed with serum samples both within and between batches of reagents.

The limitations of lipaemic samples described in the Turbitimer assay method also apply with this method, although dilution to 1:100 allowed for more lipaemic samples to be assayed with the Nephelometer than with the Turbitimer.

Limitations and interferences

The assay range was 50-650μg/l. Any samples with a result greater than 650μg/l were diluted with physiological buffered saline and re-assayed. Interference from haemolysed and icteric samples did not occur. Lipaemic samples were unable to be assayed if the analyser deemed the sample excessively turbid, despite dilution with physiological buffered saline.

3.2.6.6 TROPONIN-T ASSAY

3.2.6.6.1 PRINCIPLES UNDERLYING ASSAY PROCEDURE

An automated enzyme-immunological test for the quantitative determination of troponin-T was used for this assay (Boehringer Mannheim New Diagnostics ELISA Troponin-T).

This is a one-step enzyme-linked immunosorbent assay (ELISA). Under physiological conditions, no myocardial troponin-T is present in serum. Only after

degradation of the contractile actin-troponin complex are measurable concentrations found.

The principle of the assay is that the test sample is added to a tube coated with streptavidin. A solution containing buffer, biotinylated anti-troponin-T antibodies and troponin-T-antibody-peroxidase conjugate is then added. The incubation phase consists of any troponin-T in the sample being sandwiched between the biotinylated anti-troponin-T antibodies and the troponin-T-antibody-peroxidase conjugate. A wash phase then takes place to remove any unbound troponin-T-antibody-peroxidase conjugate. A chromogen is then added (di-ammonium 2,2'-azino-bis (3-ethylbenzo- thiazoline-6-sulphonate)) (ABTS), which reacts with the peroxidase and so produces a colour. The absorbance of this final solution is compared to a calibration curve that has been previously calculated, and the concentration of troponin-T in the original sample derived from this.

3.2.6.6.2 ASSAY PROCEDURE

All samples were brought to room temperature prior to use. Then, 200 μl of test sample and 1 ml of incubation solution were added to a tube coated with streptavidin. The incubation phase lasted 1 hour. The sample tube was washed with a washing solution, and 1 ml of substrate/chromogen solution added. A further incubation phase of 25 minutes followed, the absorbance of this final solution was then read at 422 nm.

Six standard samples of known troponin-T concentration were included in each assay run, these were analysed twice and the results obtained used to form the calibration curve. The measuring range of the assay was 0-12 ng/ml, and the lower

detection limit for myocardial damage was 0.1 ng/ml.

3.2.6.7 CREATINE KINASE ASSAY

3.2.6.7.1 PRINCIPLES UNDERLYING ASSAY PROCEDURE

This assay was performed by an automated analyser (Kodak Ektachem Clinical Chemistry Slide).

The analysis uses creatine phosphate and adenosine diphosphate (ADP) as substrates for a reaction that generates adenosine triphosphate (ATP) and creatine. Hydrogen peroxide is formed in equivalent amounts to ATP, and this oxidises a dye precursor. The rate of dye production is monitored by reflectance spectrophotometry at 670 nm. Reflectance density is measured at 37°C. The rate of change in reflection density is used to calculate enzyme activity in International Units per litre (U/L).

The reaction sequence can be summarised as follows:

Creatine phosphate + ADP	→	Creatine + ATP
ATP + glycerol	→	α-glycerophospate + ADP
α-glycerophosphate + O_2	→	Dihydroxyacetone phosphate + H_2O_2
H_2O_2 + leucodye	→	Dye + H_2O

Note: The first reaction is catalysed by creatine kinase

A peroxidase catalyses the final reaction to enable hydrogen peroxide to oxidise the dye precursor

3.2.6.7.2 ASSAY PROCEDURE

Using the principle described above (page 100), 11μl of sample was deposited on the slide, and evenly distributed due to the presence of surfactant. This spreading layer contained N-acetylcysteine which activated the creatine kinase. The reagent layer contained those components necessary for the reactions described above, the reflectance density after the final reaction was measured by the analyser. The dynamic range of the assay is 20-1600 U/L, the upper limit of normal taken as 300 U/L.

Interfering substances

Significant haemolysis is known to interfere with the assay. The nature of collection of samples in this study ensured that haemolysis of samples did not occur to interfere with particular assay in this way.

3.2.6.8 ASPARTATE AMINOTRANSFERASE ASSAY

An automated analyser was used to perform this assay (Kodak Ektachem). The analytical slide contains a number of reagents that enable the following reactions to take place when a sample of test serum is added to the slide:

Aspartate + α-ketoglutarate $\rightarrow$ oxaloacetate + glutamate

Oxaloacetate + NADH + H^+ $\rightarrow$ Malate + NAD^+

The first reaction is catalysed by aspartate aminotransferase in the presence of sodium pyridoxal-5-phosphate. A high concentration of pyridoxal-5-phosphate

allows rapid activation of aspartate aminotransferase and obviates the need for a long pre-incubation phase.

The second reaction is catalysed by malate dehydrogenase in the presence of reduced nicotinamide adenine dinucleotide (NADH). The rate of oxidation of NADH is monitored by reflectance spectrophotometry at 340 nm. The concentration of aspartate aminotransferase in the original sample is calculated from the rate of change in reflection density, in comparison with a calibration curve of known aspartate aminotransferase concentrations.

3.2.6.9 QUALITY CONTROL OF LABORATORY BASED ASSAYS

The precision of each assay was assessed by using replicate samples in individual assay runs, and the accuracy determined by using recognised control samples of known concentration. As would be expected for commercially provided assays performed on an automated analyser, both precision and accuracy were high.

BIOCHEMICAL MARKER	SD	CV (%)	CC
CK-MB ACTIVITY	6.5	4.6	0.99
MYOGLOBIN	22.6	5.2	0.95
TROPONIN-T	0.1	9.8	0.99
CREATINE KINASE	26.4	4.1	0.98
ASPARTATE AMINOTRANSFERASE	12.1	6.1	0.96

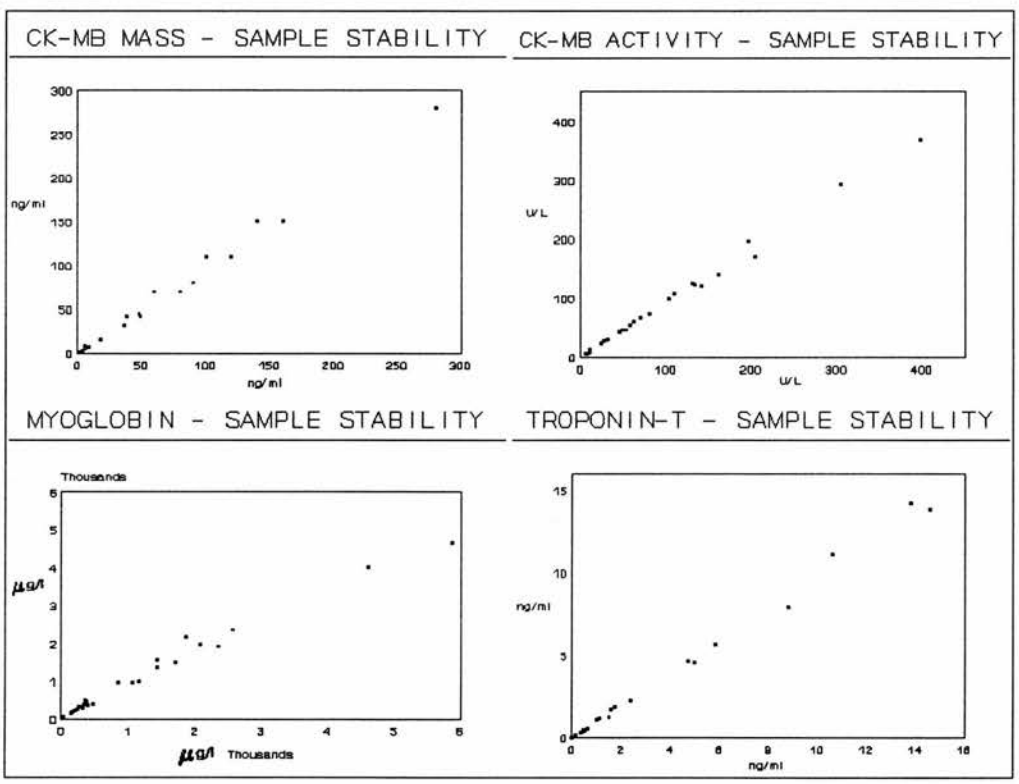
Abbreviations

SD = Standard deviation
CV = Coefficient of variation
CC = Correlation coefficient

3.2.6.9 SAMPLE STABILITY

Serum from a number of patients was retested for CK-MB mass, CK-MB activity, myoglobin and troponin-T, in more than one batch of assays to determine the stability of stored frozen samples, as well as to establish the effects of thawing and refreezing. The following figure shows the results of 2 analyses, performed 6 months apart:

Figure 1. STABILITY OF STORED SERUM SAMPLES FOR 4 MARKERS



Regression coefficients

CK-MB mass	= 0.99
CK-MB activity	= 0.99
Myoglobin	= 0.99
Troponin-T	= 0.99

3.3 DATA COLLECTION, STORAGE AND RETRIEVAL

Once collected, all data were manually recorded onto proformas, information being recorded by patient number. Data were stored on paper, and in spreadsheet format using Quattro Pro v4.0 (Borland International), on a personal computer, Viglen Genie Executive 4DX266. Statistical analysis was performed by Oxstat V, (Microsoft Corporation). Graphics were generated using Harvard Graphics v3.0, (Software Publishing Corporation). All text was written, stored and printed from Wordperfect 5.1, (Wordperfect Corporation).

3.4 STATISTICS

Serum and urine concentrations are given as mean $\pm$ standard error (SEM).

Where comparisons of 3 or more markers was made, an analysis of variance (ANOVA) was performed initially. If this showed significant differences between the variables, then paired comparisons were made by Student's t-test for paired or unpaired samples, as appropriate (Daly,1991).

Sensitivity, specificity, positive and negative predictive value were calculated by the respective formulae given in chapter 4. These indicators of diagnostic accuracy were compared by McNemar's test for comparison of paired proportions (Freedman,1987).

Diagnostic cut-offs for coronary artery reperfusion were made from receiver operating curves (ROC). The cut-off being taken as the point on each curve judged best according to ROC analysis (Hanley,1983).

Stability of stored samples was determined by simple regression analysis.

Chi-square (χ^2) test was used to compare independent sets of proportions in 2 or more groups (Daly,1991).

For all statistical analyses, differences of $p < 0.05$ were regarded as significant.

3.5 STUDY PROTOCOL

PROFORMA

A proforma was completed at the time of admission detailing the following:

Date of admission

Time of admission

Time of onset of symptoms

Pre-admission medication

Intra-muscular injection administered prior to admission to the Coronary Care Unit.

Following admission, the following information was also recorded:

Time of hook-up of ambulatory monitor

Time of 12-lead ECG recordings

Time of blood samples

Time of urine samples

Drugs administered during the study, especially, time of commencement of intra-venous thrombolytic therapy

12-LEAD ELECTROCARDIOGRAPHY

12-lead electrocardiograms were taken on admission, at 3 hourly intervals until 12 hours from the onset of symptoms, and on days 2 and 3.

AMBULATORY MONITORING

A Reynolds "Tracker" ambulatory monitor was hooked-up to the patient at the time of admission and a continuous tape recording made for 24 hours.

BLOOD SAMPLES

Twenty millilitre blood samples were taken on admission, at 2 hourly intervals until 6 hours from the onset of symptoms, and at 3 hourly intervals until 12 hours from the onset of symptoms.

URINE SAMPLES

A 20 millilitre urine sample was collected as soon as possible after admission, and all subsequent urine voided thereafter until 12 hours from the onset of symptoms.

CHAPTER 4 - RESULTS:

BIOCHEMICAL DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

CHAPTER 4 - RESULTS: BIOCHEMICAL DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION **PAGE**

4.1 BIOCHEMICAL DIAGNOSIS AND DIFFERENTIATION OF ACUTE MYOCARDIAL INFARCTION

4.1.1 PATIENT CHARACTERISTICS

One hundred and ninety one patients were recruited into the study. All patients, or a relative, gave informed consent at the time of entry. Patients were divided into certain categories on the basis of the admission ECG. The admission ECG was used as the discriminating factor because it was readily available for all patients, and because it is independent of the biochemical markers. Separation of patients into groups must be performed without using biochemical criteria to enable a valid comparison of the biochemical markers (Freedman, 1987). Analysis of the admission ECG was performed independently of all other data, and the division of patients into the following groups was made before analysis of the biochemical data.

1) Group A - ECG on admission diagnostic of acute myocardial infarction, using the following recognised ECG criteria:

ST segment elevation $\geq$ 0.1mV in 2 or more adjacent limb leads

ST segment elevation $\geq$ 0.2mV in 2 or more adjacent chest leads

R wave in lead V1 $>$ 1/4 height of ensuing S wave

2) Group B - ECG on admission not diagnostic of AMI but AMI proven at day 3 by accepted biochemical criteria (in accordance with the World Health Organisation guidelines (WHO,1962)). In our laboratory these were:

Creatine kinase $>$ 2x upper limit of normal (300 U/L)

Aspartate aminotransferase $>$ 2x upper limit of normal (80 U/L)

3) Group C - ECG on admission non-diagnostic of AMI and biochemical changes at day 3 non-diagnostic of AMI.

To allow comparison between QAMI and NQAMI, those patients identified as having sustained AMI (Groups A and B) were also divided into either of the following 2 types:

1) Type 1 - ECG on day 3 positive for Q waves, defined as the presence of Q waves in 2 or more adjacent limb leads or 2 or more adjacent pre-cordial leads, a Q wave having to be at least 1/4 the height of the ensuing R wave, and greater than 0.04 seconds in duration.

Note: If Q waves were present on the admission ECG and the Q wave distribution was unchanged on the day 3 ECG, patients were still assigned to this group.

2) Type 2 - ECG on day 3 negative for Q waves as defined above.

It follows that only patients without Q waves on any ECG in the first 3 days after admission qualified for this group.

The following table lists the patient characteristics for all patients and sub-groups:

Table 1. PATIENT CHARACTERISTICS

Patient Group	Number	Males	Age range (years)	Mean age (years)
All patients	191	116	37-86	62.6
All infarcts	104	72	37-86	64.2
Group A	56	41	37-84	64.9
Group B	48	31	40-86	63.3
Group C	87	43	37-78	60.5
Type 1	58	46	37-84	64.4
Type 2	46	26	40-86	63.6
Males	116	116	37-84	61.4
Females	75	0	42-86	64.8
Group A males	41	41	37-84	63.9
Group A females	15	0	48-86	67.6
Group B males	31	31	40-79	61.6
Group B females	17	0	42-78	66.4
Type 1 males	46	46	37-83	63.8
Type 1 females	12	0	48-74	66.9
Type 2 males	26	26	40-86	61.0
Type 2 females	20	0	42-86	67.0

Of those patients in groups A and B, the subdivision into types 1 and 2 was:

	Type 1	Type 2	Total
Group A	43	13	56
Group B	14	34	48
Total	58	46	104

Age differences between patient groups

Analysis of the means of the above groups by analysis of variance (ANOVA) gave a variance ratio of 2.10 with 688 total degrees of freedom. This was statistically significant, $p<0.05$. Differences between the mean ages for the following comparisons were identified, (Student's t-test for means of unpaired samples), mean ages in brackets:

Group A (64.9)	vs	Group C (60.5)	p=0.015
Type I (64.4)	vs	Group C (60.5)	p=0.026
Females (64.8)	vs	Males (61.4)	p=0.02
Type 2 females (67.0)	vs	Type 2 males (61.0)	p=0.02
Group B females (66.4)	vs	Group B males (61.6)	p=0.062

4.1.1.1 DISCUSSION

Age distribution in patient groups

Patients in group A were older than patients in group C, as were patients with type 1 infarction. This combination is not surprising since 77% of patients in group A had type 1 infarcts, and patients with infarction would be expected to be older than a control population admitted to CCU with chest pain of presumed cardiac cause. Generally, females were older than males, and specifically, females with type 2 infarction were significantly older than males with type 2 infarcts. This has been shown previously (Willich,1987). There was a trend for females in group B to be older than males, p=0.06.

Sex distribution in patient groups

A comparison of sex distribution in the various groups revealed a significant preponderance of females in group C, $\chi^2 = 10.16$, $p < 0.005$. There was also a larger than expected proportion of female patients with type 2 infarction, $\chi^2 = 6.16$, $p < 0.01$. Neither of these results is unexpected as these observations have been made previously, but they confirm that patient demographics in this study were similar to those documented in previous studies of patients with chest pain suggestive of AMI admitted to a coronary care unit.

Infarct type

Analysis of the distribution of infarct type in groups A and B revealed an excess of patients with type 1 infarction in group A, and type 2 infarction in group B, $\chi^2 = 23.64$, $p < 0.005$. That is, patients with an admission ECG which is diagnostic of AMI are more likely to have QAMI, whereas patients with a non-diagnostic admission ECG are more likely to have NQAMI.

The total proportion of patients with NQAMI was 46%. This is higher than the figure reported in most studies. This can be explained by the strict ECG criteria used to classify patients into type 1 and type 2 infarction, namely, the requirement of a Q-wave to be greater than 1/4 the height of the ensuing R wave, and to be at least 0.04 seconds in duration.

All patients with chest pain suggestive of a cardiac aetiology were recruited into the study, patients were not excluded on the basis of a previous cardiac history. The day 3 ECG was chosen as the reference point for classification into type 1 or type 2 because, as discussed above, Q-waves can be transient in the first few days

after AMI. Although it could be argued that if Q-waves are present in the inferior leads on admission, and are present in the same leads at day 3 that these are not "new", this is not guaranteed to be so. Consequently, the presence of Q-waves on the day 3 ECG as defined by the above criteria and used in this study is precise. This ensured that subjective interpretation of deciding whether the presence of Q-waves at day 3 is related to the current, or previous, infarction was removed from ECG analysis.

It is also important to note that the separation of patients into infarct types was performed prior to information being available from the biochemical analysis, and that this ECG classification was used, without variation, for all subsequent comparisons between the patient groups for the various biochemical markers being evaluated.

4.1.2 DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION IN INDIVIDUAL PATIENT GROUPS

One of the primary endpoints of this project was to evaluate the capability of serum and urine creatine concentrations to diagnose acute myocardial infarction. The other biochemical markers measured are recognised to be of value for this purpose, but their ability to differentiate between the various patient groups as detailed above, is not well known.

This section compares differences in serum and urine concentrations for each individual marker for the various patient groups.

4.1.2.1 PEAK SERUM AND URINE CONCENTRATIONS WITHIN 12 HOURS OF THE ONSET OF SYMPTOMS

The period of 12 hours from the onset of symptoms was chosen as the period of assessment. This is in accordance with current understanding of the time window in which thrombolytic therapy is likely to be of clinical benefit.

To compare serum and urine concentrations between the groups, the peak concentration within this period was chosen as the "representative" figure for analysis. Further analysis of serum concentrations at different time intervals following the onset of symptoms is performed later in this chapter. Tables 17-21, pages 150-163 list, by patient group, peak concentrations within 12 hours of symptom onset. The means of peak serum or urine concentration were assessed initially by ANOVA. If a significant difference was detected, then the various groups were compared to one another, in pairs, using an independent t-test. The following tables and figures show the results for the various markers.

4.1.2.1.1 RESULTS: CREATINE AND CREATININE

Table 2. MEAN SERUM CREATINE CONCENTRATIONS

Patient group	Mean concentration (μmol/l)	S.E.M.
All infarcts	70.86	3.67
Group A	65.89	3.99
Group B	76.68	6.39
Group C	74.64	4.32
Type 1	67.14	4.08
Type 2	74.80	6.35

One way ANOVA, variance ratio=0.774, P=NS

Figure 2. SERUM CREATINE CONCENTRATIONS

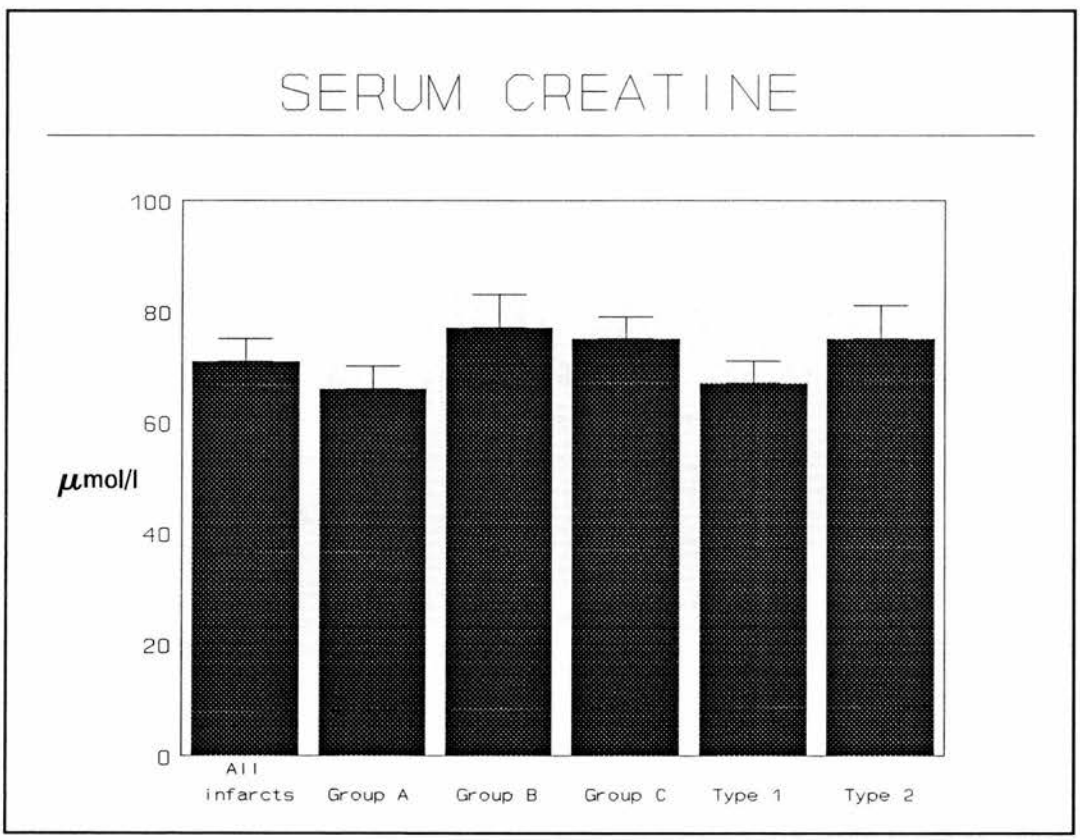
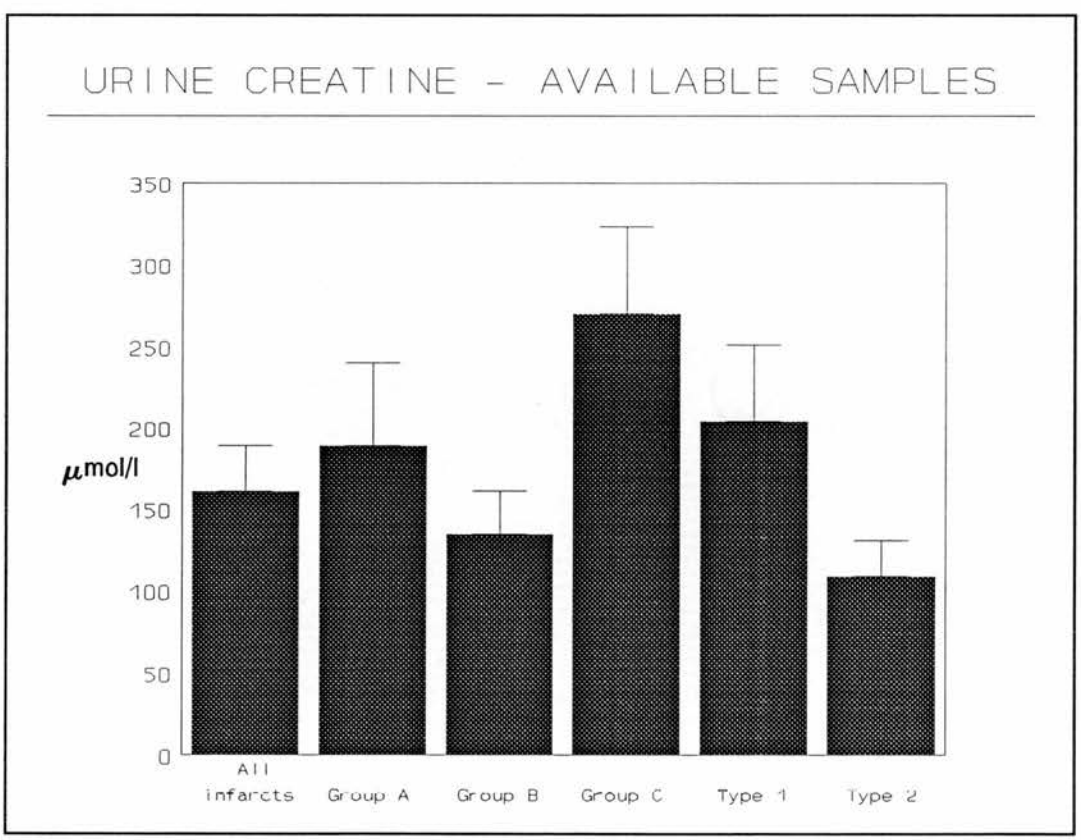


Table 3. URINE CREATINE CONCENTRATIONS - Available samples

Patient group	Mean concentration (μmol/l)	S.E.M.
All infarcts	160.78	27.77
Group A	189.20	50.92
Group B	134.89	25.84
Group C	269.63	52.80
Type 1	204.11	47.42
Type 2	109.27	22.1

One way ANOVA, variance ratio=1.96, P=NS

Figure 3. URINE CREATINE CONCENTRATIONS - Available samples



A number of patients were unable to provide a sample of urine for analysis, they were divided amongst the groups as follows:

Table 4. INABILITY TO PROVIDE A SAMPLE OF URINE BY PATIENT GROUP

Patient group	**Urine passed within 12 hours of symptom onset (n)**	**No urine passed within 12 hours of symptoms (n)**
Group A	44	12
Group B	45	3
Group C	74	13
Type 1	48	10
Type 2	41	5

A comparison of the groups by chi-squared test revealed a significant difference in inability to pass urine between groups A and B, $\chi^2=4.77$, $p<0.05$. There was no significant difference found between all patients with AMI and group C, $\chi^2=0.007$, or between type 1 and type 2 infarction, $\chi^2=0.81$.

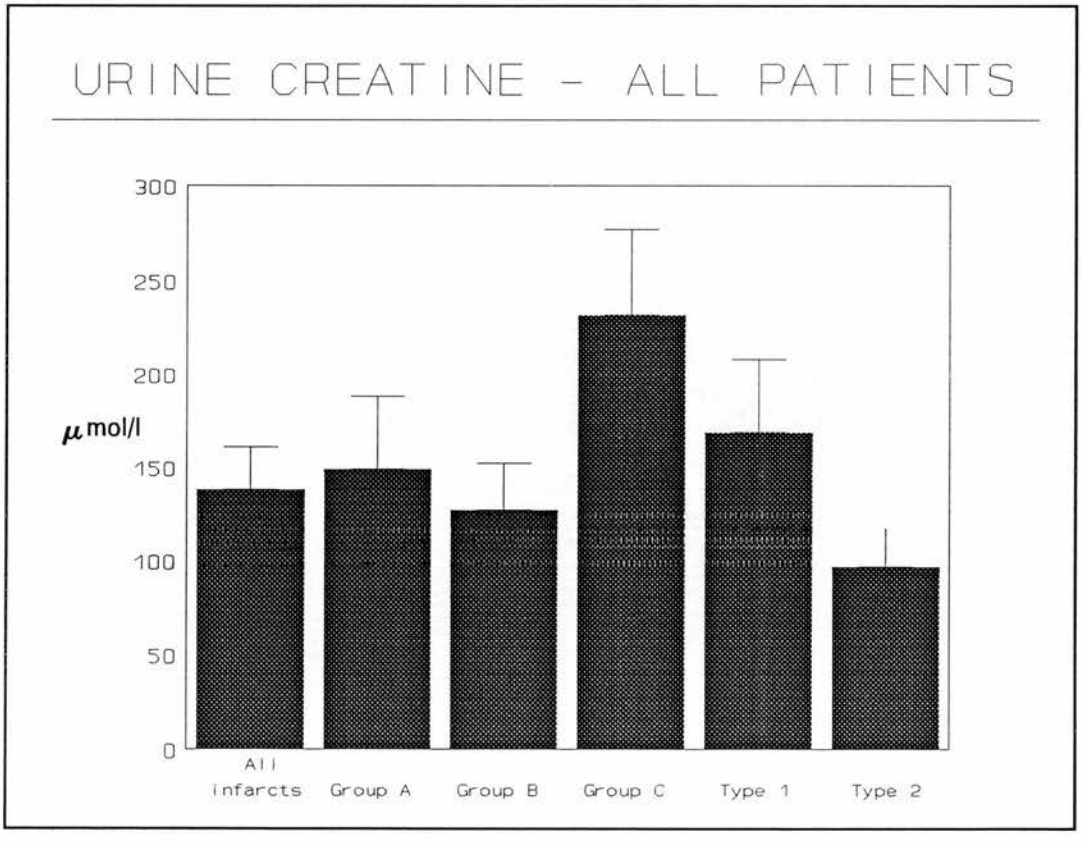
Since urine creatine concentrations were being assessed for their ability to diagnose AMI, it is inappropriate not to include all patients studied. Thus, if a urine sample is not provided, such a patient should not be excluded from the analysis as this may bias the results (Harris,1981). Consequently, if a value of 0μmol/l was substituted for those patients who were unable to provide a sample of urine within 12 hours of symptom onset, the results for peak urinary concentration were as follows:

Table 5. MEAN URINE CREATINE CONCENTRATIONS - All patients

Patient group	Mean concentration (μmol/l)	S.E.M.
All infarcts	138.4	23.6
Group A	148.7	38.6
Group B	126.5	24.7
Group C	232.4	44.8
Type 1	168.9	38.9
Type 2	97.4	20.3

One way ANOVA, variance ratio=1.79, p=NS

Figure 4. URINE CREATINE CONCENTRATIONS - All patients



Because of the close biochemical association between creatine and creatinine, and the fact that analysis of these two substances was performed by the same assay system, albeit with a modification to measure creatine, serum and urine concentrations of creatinine were analysed in a similar manner to above.

Table 6. MEAN SERUM CREATININE CONCENTRATIONS

Patient group	**Mean concentration (μmol/l)**	**S.E.M.**
All infarcts	105.3	37.4
Group A	109.6	42.7
Group B	100.3	29.8
Group C	99.4	26.8
Type 1	105.8	32.9
Type 2	103.7	43.1

One way ANOVA, variance=0.72, p=NS

Figure 5. SERUM CREATININE CONCENTRATIONS

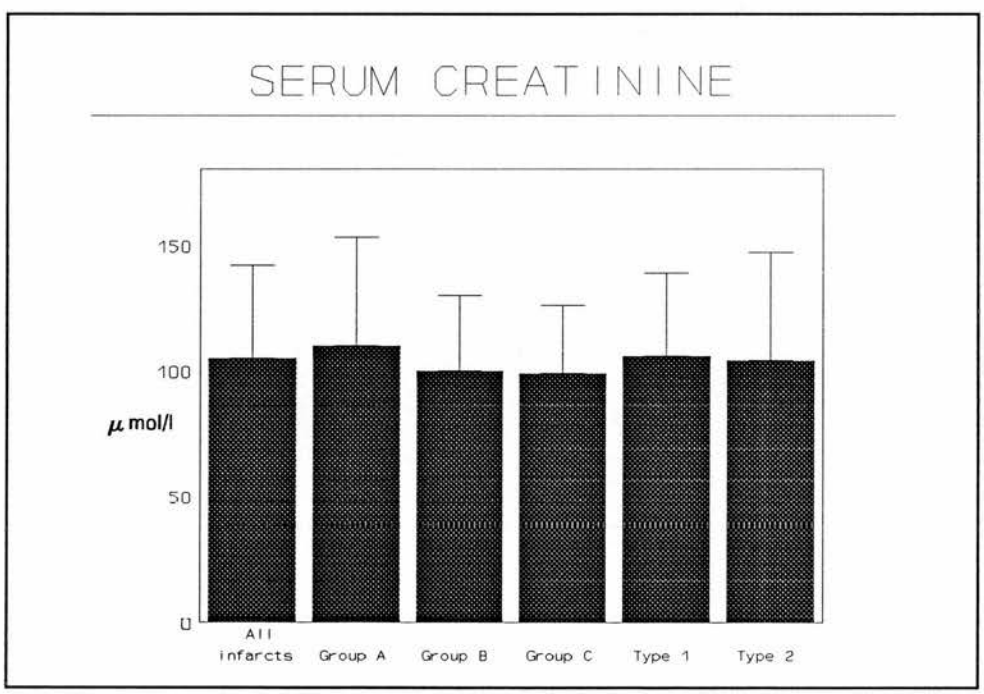


Table 7. URINE CREATININE CONCENTRATIONS - Available samples

Patient group	Mean concentration (μmol/l)	S.E.M.
All infarcts	8345	643
Group A	9677	996
Group B	7042	780
Group C	5349	407
Type 1	8984	945
Type 2	7596	849

One way ANOVA, variance=4.61, $p<0.01$

Figure 6. URINE CREATININE CONCENTRATIONS - Available samples

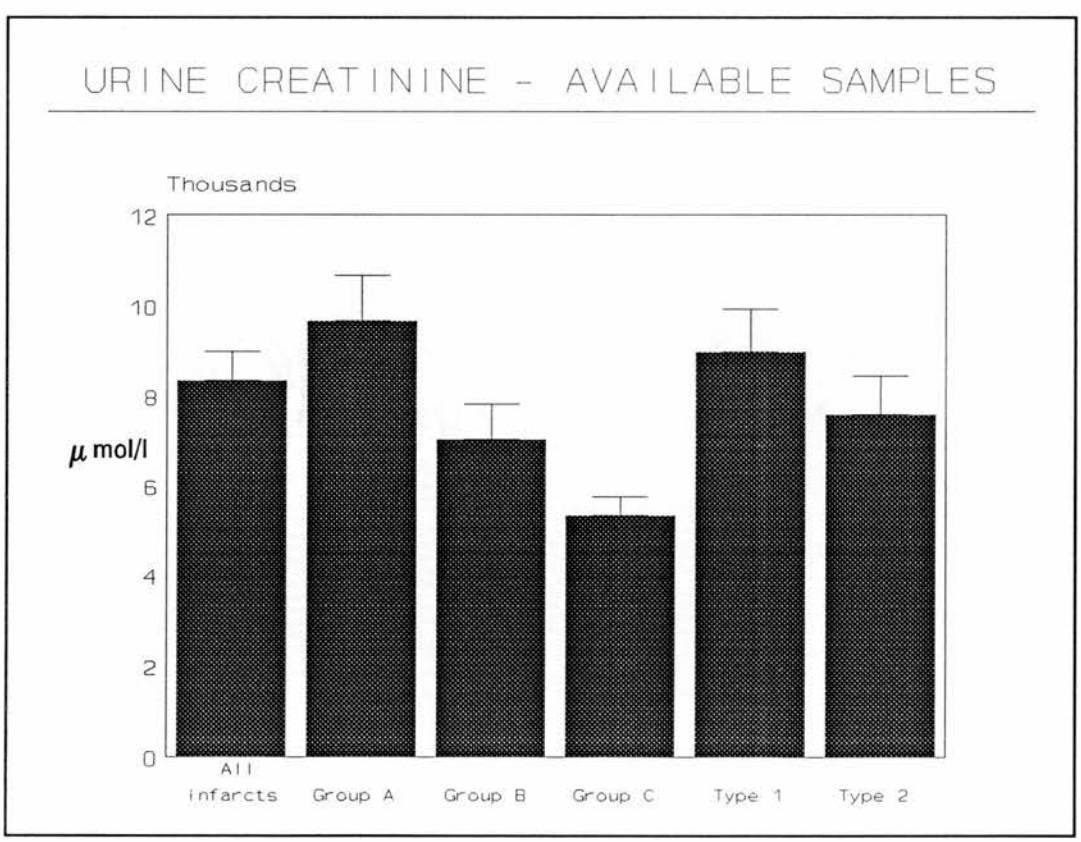
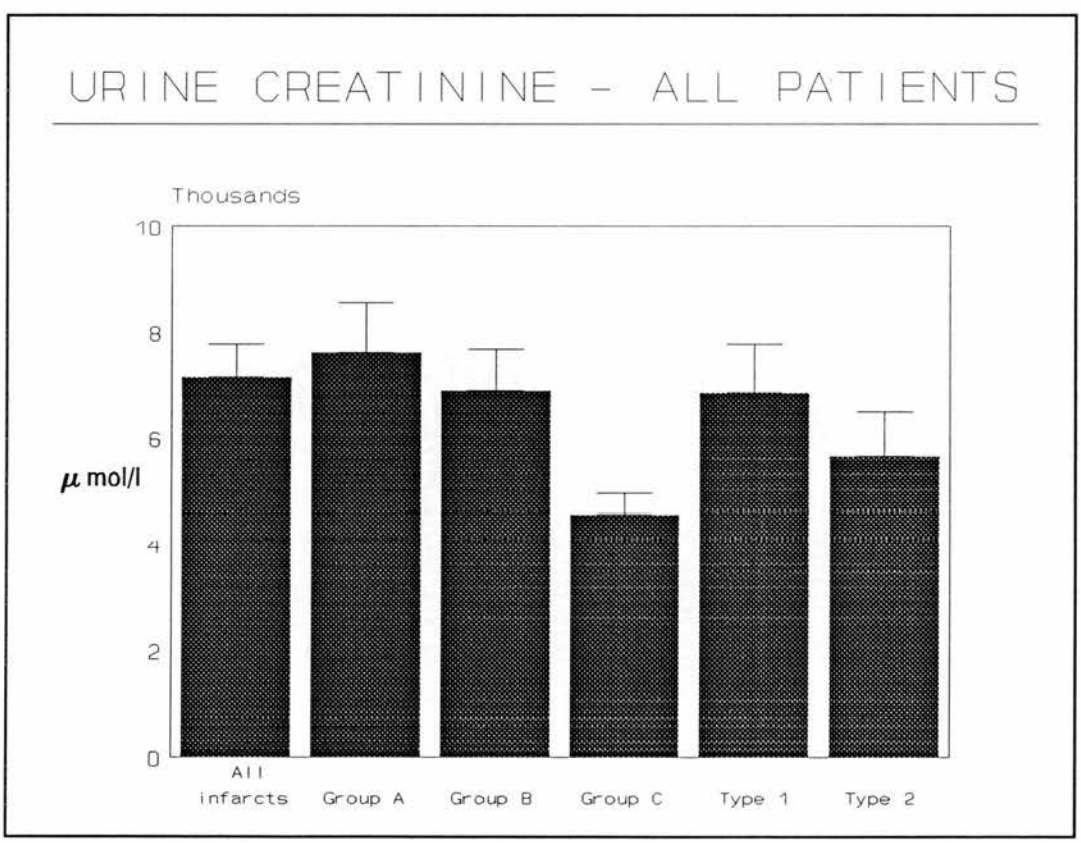


Table 8. URINE CREATININE CONCENTRATIONS - All patients

Patient group	Mean concentration (μmol/l)	S.E.M.
All infarcts	7141	621
Group A	7603	947
Group B	6889	778
Group C	4550	402
Type 1	6858	901
Type 2	5656	834

One way ANOVA, variance=2.89, $p<0.05$

Figure 7. URINE CREATININE CONCENTRATIONS - All patients



For urine creatinine concentrations in all patients the following significant differences were identified:

Groups compared	t-statistic	p-value
Group C All infarcts	3.35	<0.001
Group C Group A	3.36	0.001
Group C Group B	2.96	0.004
Group C Type 1	3.26	0.001
Group C Type 2	2.71	0.008

4.1.2.1.1.1 DISCUSSION

These results show that within 12 hours of the onset of symptoms, neither serum nor urinary creatine concentrations were able to differentiate any group with AMI from control subjects. The range of serum concentrations was especially low. Although patients in group B and type 2 had higher mean concentrations than patients in group A and type 1, this was reversed for urinary concentrations, with group A and type 1 having higher means than group B and type 2. From above (page 81), it was proposed on theoretical grounds that urinary creatine concentrations should reflect serum concentrations but with a concentrating effect, clearly this was not apparent in this study population. Problems with using urine as a diagnostic test because of its unavailability in some patients have been noted previously (Suval,1987). Lack of sample availability was observed in all 3 patient groups, although this was only significantly different between groups A and B, the

former having more anuric patients up to 12 hours after symptoms had begun. This would be expected, because these patients have larger infarcts with increased haemodynamic compromise.

Why such a discrepancy exists between this study and that of DeLanghe et al is unclear (DeLanghe,1988). Theirs is the only study in the literature which reports the value of creatine as a diagnostic marker of AMI. All patients in this study had large infarcts, and the control population was "normal" subjects, not an equivalent group of hospitalised patients. Of the 22 patients with AMI, 12 had bladder and/or arterial catheters inserted, the mean CK rise was over 3000U/L. The mean serum concentration in their control population was approximately 45μmol/l, this was lower than the mean concentration of patients in group C, 74.6μmol/l. The effects of stress on serum and urine creatine levels are not known, but the metabolic role of creatine may lead to it being affected by an increase in circulating catecholamine levels at the time of admission to CCU, irrespective of the aetiology of chest pain, as for patients in group C.

There was no close correlation between either serum, or urine concentrations of creatine and creatinine, so confirming that the assays used to measure these 2 substances were different. The quality control data for the 2 assays showed their respective accuracy in the measurement of these 2 quantities, therefore it would appear to be justified to state that creatine and creatinine were assayed separately and specifically. There were no significant differences between the groups for serum creatinine concentrations, and although urine concentrations were different, this reflected the type of infarction, namely urine was more concentrated for patients with larger infarcts (group A and type 1), than patients with smaller infarcts (group

B and type 2), than patients without infarction, (group C). It follows that the unavailability of urine was the same for creatinine as for creatine, and since this was evident for 28 of the 191 patients in the study, the usefulness of urine creatinine in any diagnostic capacity is severely limited and as such it can have no role as a diagnostic marker in the early post-AMI period.

Now, serum CK-MB mass, serum CK-MB activity, serum myoglobin and serum troponin-T concentrations will be considered.

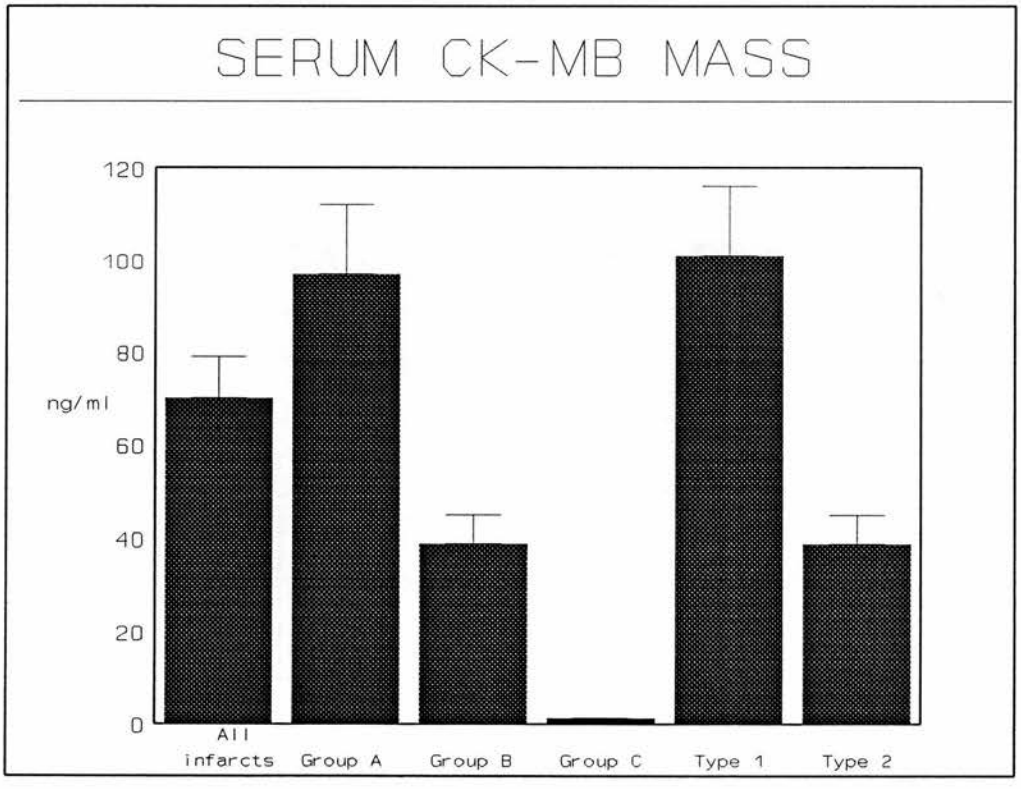
4.1.2.1.2 RESULTS: CK-MB MASS, CK-MB ACTIVITY, MYOGLOBIN AND TROPONIN-T

Table 9. MEAN SERUM CK-MB MASS CONCENTRATIONS

Patient group	Mean concentration (ng/ml)	S.E.M.
All infarcts	69.97	8.91
Group A	96.59	14.93
Group B	38.92	5.88
Group C	1.34	0.10
Type 1	101.57	15.34
Type 2	38.63	6.05

One way ANOVA, variance ratio=16.58, $p<0.01$.

Figure 8. SERUM CK-MB MASS CONCENTRATIONS



The following table shows the significant differences identified between pairs of groups.

Table 10. SERUM CK-MB MASS - GROUP COMPARISONS

Groups compared	**t-statistic**	**p-value**
Group C All infarcts	7.04	<0.001
Group C Group A	7.97	<0.001
Group C Group B	8.62	<0.001
Group C Type 1	8.02	<0.001
Group C Type 2	8.51	<0.001
Group A Group B	3.59	<0.001
Type 1 Type 2	3.49	<0.001

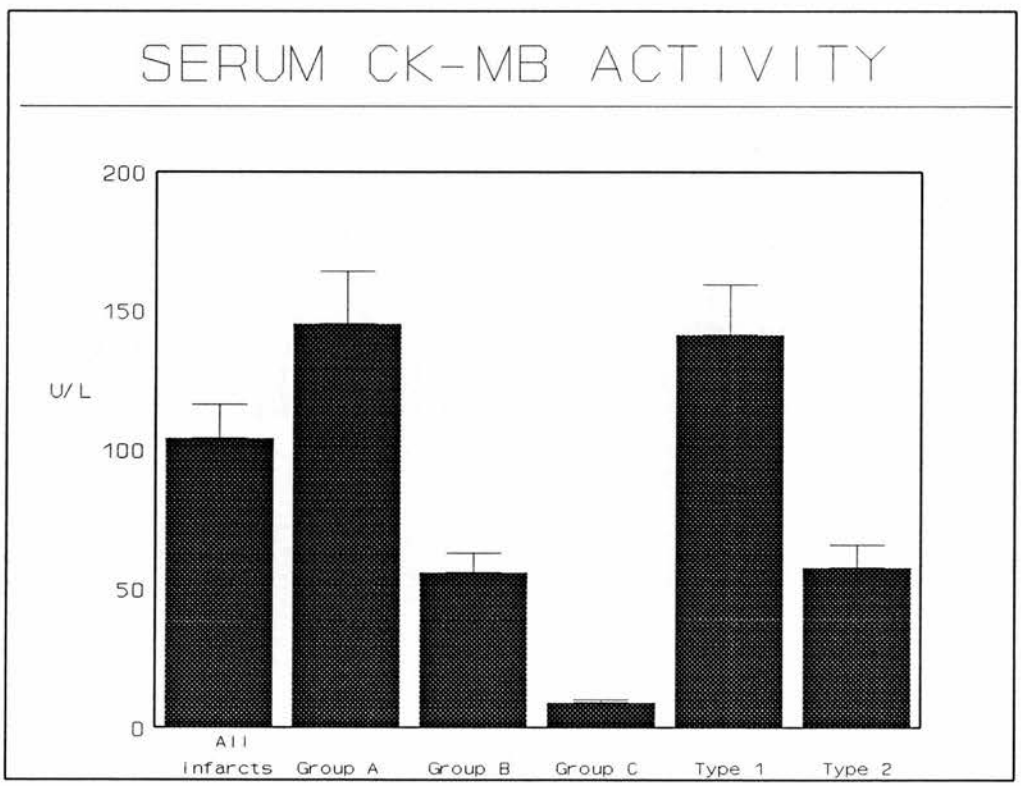
These results are discussed at the end of this section (page 147)

Table 11. MEAN SERUM CK-MB ACTIVITY

Patient group	Mean concentration (U/L)	S.E.M.
All infarcts	103.81	11.74
Group A	144.5	19.32
Group B	56.3	7.49
Group C	8.95	0.42
Type 1	140.53	18.72
Type 2	57.59	8.26

One way ANOVA, variance ratio=19.18, $p<0.01$

Figure 9. SERUM CK-MB ACTIVITY CONCENTRATIONS



The following table shows the significant differences identified between pairs of groups.

Table 12. SERUM CK-MB ACTIVITY - GROUP COMPARISONS

Groups compared	**t-statistic**	**p value**
Group C All infarcts	7.38	<0.001
Group C Group A	8.76	<0.001
Group C Group B	8.50	<0.001
Group C Type 1	8.62	<0.001
Group C Type 2	8.10	<0.001
Group A Group B	4.01	<0.001
Type 1 Type 2	3.72	<0.001

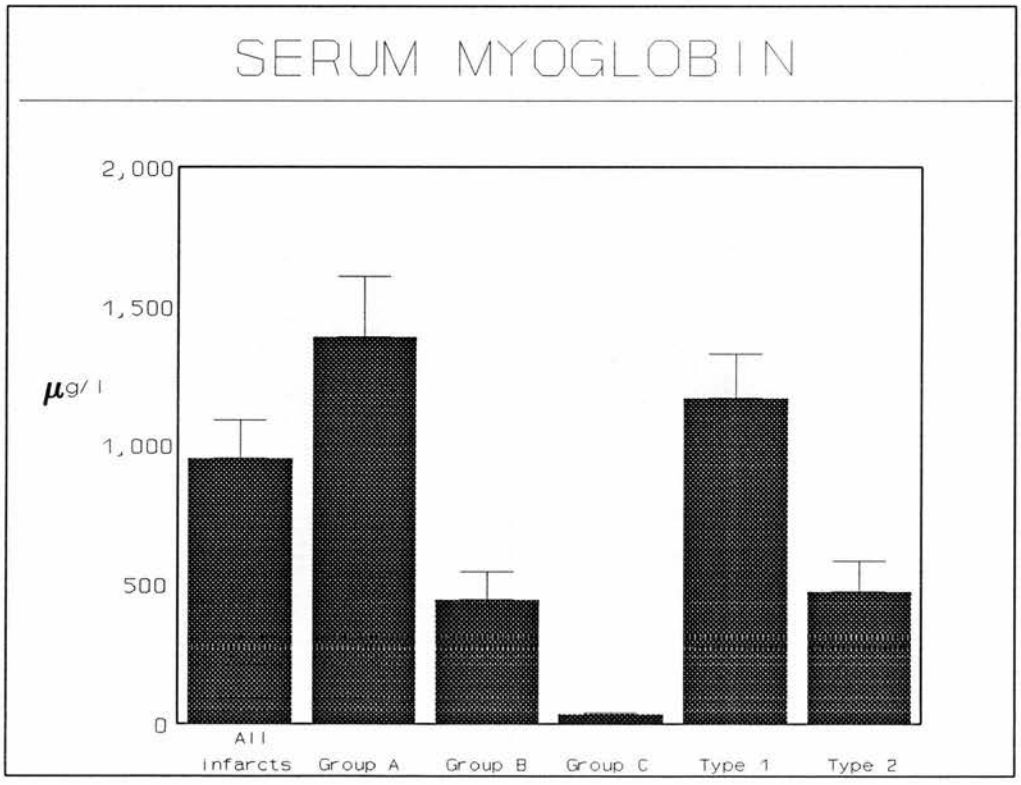
These results are discussed at the end of this section (page 147).

Table 13. MEAN SERUM MYOGLOBIN CONCENTRATIONS

Patient group	Mean concentration (μg/l)	S.E.M.
All infarcts	953.64	134.87
Group A	1390.13	219.69
Group B	444.42	101.16
Group C	38.44	3.03
Type 1	1168.34	159.05
Type 2	476.34	107.99

One way ANOVA, variance ratio=14.78, $p<0.01$.

Figure 10. SERUM MYOGLOBIN CONCENTRATIONS



The following table shows the significant differences identified between pairs of groups.

Table 14. SERUM MYOGLOBIN - GROUP COMPARISONS

Groups compared	**t-statistic**	**p value**
Group C All infarcts	6.20	<0.001
Group C Group A	7.68	<0.001
Group C Group B	5.41	<0.001
Group C Type 1	8.71	<0.001
Group C Type 2	5.59	<0.001
Group A Group B	3.71	<0.001
Type 1 Type 2	3.41	<0.001

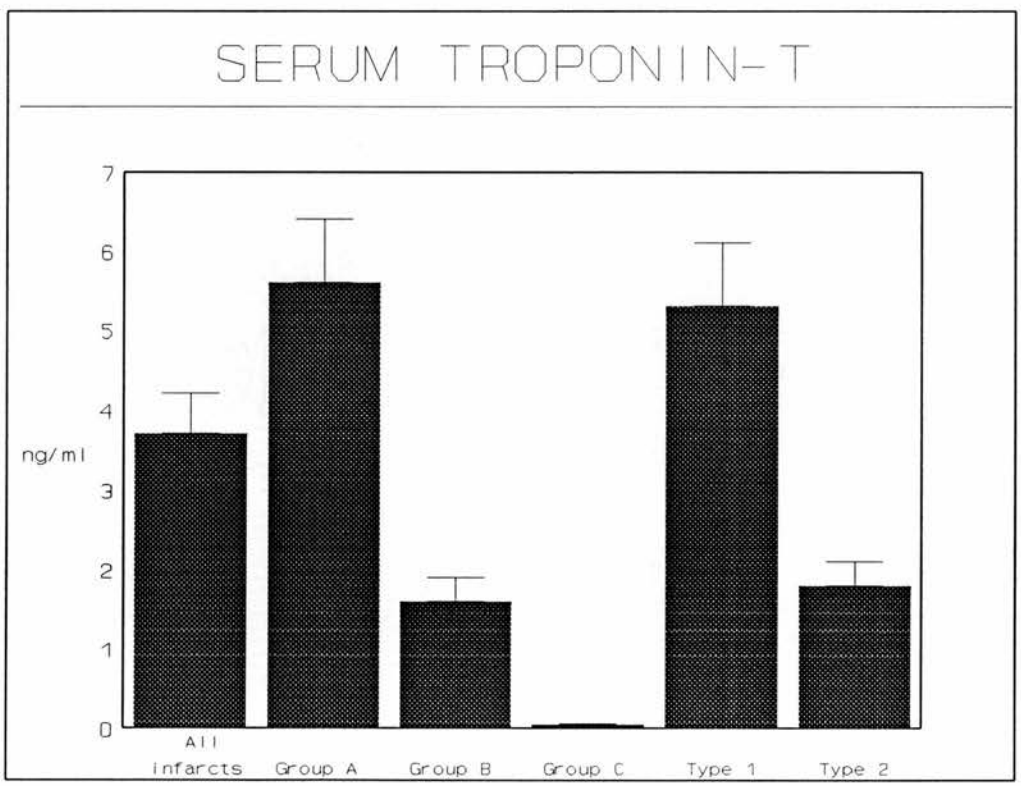
These results are discussed at the end of this section (page 147).

Table 15. MEAN SERUM TROPONIN-T CONCENTRATIONS

Patient group	Mean concentration (ng/ml)	S.E.M.
All infarcts	3.74	0.48
Group A	5.62	0.79
Group B	1.56	0.28
Group C	0.05	0.01
Type 1	5.31	0.77
Type 2	1.76	0.34

One way ANOVA, variance ratio=19.49, $p<0.001$.

Figure 11. SERUM TROPONIN-T CONCENTRATIONS



The following table shows the significant differences identified between pairs of groups.

Table 16. SERUM TROPONIN-T - GROUP COMPARISONS

Groups compared	t-statistic	p value
Group C All infarcts	6.98	<0.001
Group C Group A	8.85	<0.001
Group C Group B	7.23	<0.001
Group C Type 1	8.42	<0.001
Group C Type 2	6.96	<0.001
Group A Group B	4.58	<0.001
Type 1 Type 2	3.89	<0.001

4.1.2.1.2.1 DISCUSSION

The results for the 4 markers, CK-MB mass and activity, myoglobin and troponin-T are all similar. Analysis of variance on the means of all groups shows significant differences between them. Comparing the groups in pairs also shows similar findings for all 4 markers. There are differences between group C and all patients with infarction (groups A and B combined), as well as between group C and the 4 sub-groups individually, namely groups A and B, and types 1 and 2. It would be expected that significant differences would be seen between patients with and without infarction. Similarly, all 4 markers would be expected to discriminate group

C from all sub-groups of AMI, as they have all been shown to be selective for AMI in numerous previous studies.

What is of interest is that the peak serum concentration within 12 hours of the onset of symptoms is able to differentiate between patients in the various groups. Thus it can discriminate between patients with and without diagnostic ECGs on admission (groups A and B), as well as those patients who do or do not develop Q-waves by the third day after infarction (types 1 and 2). Comparison of group A with type 1, and group B with type 2 was not statistically significant for any marker, conversely, comparison of group A with type 2, and group B with type 1 gave similar results to those listed for comparison of group A with group B and type 1 with type 2. This is expected because of the considerable overlap between group A and type 1, and group B and type 2. The number of patients in this study was too small to identify any differences between these latter particular types of patients. It may be that with larger numbers differences would be identified, although the overlap noted between group A and type 1 in this study would be expected to persist in any similar patient population.

In terms of stratifying patients and commencing appropriate treatment as soon as possible after the onset of symptoms, these results are of potential clinical significance. Firstly, they show overwhelmingly that patients with non-diagnostic ECG's can be identified by a number of markers soon after the onset of infarction. Secondly, they show that from a biochemical perspective certain patient groups appear to diverge at a very early stage, and as such can be distinguished by biochemical criteria within 12 hours of symptom onset. Admittedly, in this study the classification of patient groups was made retrospectively, and importantly, the

diagnosis of infarction was made using biochemical markers that are now increasingly recognised as having being superseded by the markers under evaluation in this study (although creatine kinase and aspartate aminotransferase are still the 2 most commonly used markers of infarction employed in clinical chemistry laboratories in the UK). The above findings, and those described later, should be tested in a prospective fashion with the cut-offs for differentiation of patient groups established prior to patient recruitment.

However, as the management of patients with AMI becomes increasingly varied, the recognition that early identification of patient subgroups can be achieved by biochemical means raises the possibility of tailoring the immediate post-AMI management to the specific type of infarction sustained. As indicated above, further studies will be required to assess the effects on prognosis of stratification of treatment in this way.

Table 17. Peak serum and urine concentrations within 12 hours of onset of symptoms for patients in group A.

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (Serum)	Creatine (Urine)
9	90	111	1311	0.56	40	63
10	50	72	1191	1.36	50	NIL
15	260	319	640	17.60	50	NIL
17	240	313	1611	16.30	44	62
26	70	113	356	13.40	33	81
32	400	442	718	18.20	65	185
33	180	240	2160	11.60	96	859
34	26	36	305	1.99	73	108
36	60	92	514	1.85	30	112
40	80	110	658	1.51	71	34
42	100	149	1608	13.10	45	78
49	27	24	104	1.10	71	42
53	240	285	3260	15.70	62	267
59	700	883	4986	21.30	67	NIL
63	39	70	361	6.80	62	54
66	130	193	409	4.80	62	14
71	16	45	352	0.70	53	30
80	110	157	1062	1.50	67	33
82	130	200	5391	3.40	36	NIL
83	46	73	537	6.90	65	NIL
85	80	106	1920	5.80	38	10
89	22	41	272	0.30	65	326
91	90	144	1050	5.74	75	26
92	<2	7	24	0.33	97	162
96	2	15	65	0.92	72	169
99	140	240	934	9.72	49	221

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (Serum)	Creatine (Urine)
108	<2	22	95	0.25	54	40
109	60	100	805	1.02	50	NIL
111	100	148	2490	1.04	62	13
115	42	75	1245	3.45	30	NIL
121	38	23	321	0.59	70	NIL
123	32	43	478	0.55	33	77
127	60	85	1350	4.54	85	11
135	280	397	2570	14.70	36	28
136	90	134	1860	10.60	81	NIL
138	6	45	403	1.60	40	59
141	18	32	294	1.15	39	NIL
142	110	149	1280	12.90	62	144
147	60	82	489	2.28	113	364
149	120	161	1440	4.73	73	NIL
150	160	196	2890	5.83	178	1723
154	100	141	2360	1.10	11	225
155	70	102	174	4.18	59	57
157	4	74	540	0.73	61	53
159	18	75	1370	0.33	63	20
161	320	412	4060	8.44	122	112
163	50	81	468	2.89	71	NIL
164	60	92	318	1.55	120	1045
167	150	221	2300	16.50	62	402
169	36	51	112	4.10	53	NIL
171	15	25	65	0.62	56	23
173	130	197	2520	1.69	60	NIL
187	210	265	2760	13.44	92	393
191	2	14	408	0.12	62	NIL

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (Serum)	Creatine (Urine)
193	90	126	740	9.82	64	21
197	8	43	343	1.42	71	22

ABBREVIATIONS

CK-MB mass = Serum concentration of CK-MB mass in ng/ml

CK-MB activity = Serum concentration of CK-MB activity in U/L

Myo = Serum concentration of myoglobin in μg/l

Troponin = Serum concentration of troponin-T in ng/ml

Creatine (serum) = Serum concentration in μmol/l

Creatine (urine) = Urine concenbtration in μmol/l

Table 18. Peak serum and urine concentrations within 12 hours of onset of symptoms for patients in group B.

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
1	50	57	419	2.47	37	21
4	19	25	144	0.05	133	4
7	90	120	855	2.09	95	492
13	19	19	113	0.11	44	23
27	33	58	226	2.71	65	185
29	11	21	60	0.30	67	174
31	70	79	322	0.53	48	256
35	80	115	1740	2.09	44	137
39	<2	10	308	0.84	74	44
44	10	22	26	0.79	55	29
47	8	21	24	0.45	28	36
52	80	121	105	1.70	72	51
54	20	39	282	8.94	39	37
57	13	21	78	0.37	159	88
60	18	17	212	0.17	102	337
64	42	35	678	3.99	54	15
65	41	33	137	0.48	65	242
67	8	10	55	0.32	119	36
73	220	267	4487	2.52	137	73
74	2	10	24	0.25	45	25
76	12	25	55	0.50	73	323
78	6	14	1092	0.23	228	NIL
81	18	31	106	1.86	160	31
94	41	79	189	0.58	73	34
95	50	86	616	7.39	55	76
104	26	43	287	0.66	98	36

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
113	9	21	138	0.25	74	15
116	34	59	122	3.39	91	NIL
118	6	10	108	0.61	48	54
120	39	53	636	2.28	64	33
122	43	23	144	0.43	102	47
125	170	215	226	1.95	61	98
129	8	23	112	6.90	53	44
132	90	145	1360	4.20	232	562
134	22	23	107	0.45	84	53
144	80	104	728	1.65	29	204
148	25	25	340	0.94	52	46
153	40	66	782	0.23	49	509
168	29	63	201	0.99	50	8
172	36	94	607	0.51	45	47
176	44	72	227	0.91	91	692
179	18	42	850	0.22	76	340
180	48	47	739	2.87	56	15
183	44	86	584	0.24	32	45
184	6	14	50	0.13	49	15
186	33	48	158	1.20	118	450
189	21	49	336	1.59	75	7
195	35	48	138	0.38	64	12

Table 19. Peak serum and urine concentrations within 12 hours of symptom onset for patients in group C.

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
2	<2	9	24	0.00	58	59
3	<2	4	24	0.00	44	38
5	<2	6	101	0.00	113	75
6	3	12	129	0.00	121	82
8	<2	15	24	0.00	48	71
11	<2	13	24	0.02	67	268
14	<2	10	24	0.01	69	749
16	<2	8	24	0.03	57	147
18	<2	13	24	0.04	55	13
19	<2	6	24	0.03	84	28
20	<2	6	24	0.00	69	94
21	3	8	24	0.02	29	17
22	<2	15	24	0.07	56	19
23	<2	10	24	0.00	98	171
28	<2	7	24	0.09	75	590
30	<2	8	24	0.06	36	67
37	<2	7	24	0.10	84	401
38	<2	8	24	0.09	42	22
41	<2	6	76	0.15	78	48
43	<2	6	24	0.18	50	29
45	<2	6	24	0.14	103	786
46	2	18	96	0.13	219	46
48	<2	6	24	0.12	292	951
50	<2	8	24	0.10	57	817
51	<2	5	24	0.10	16	114
55	2	10	24	0.08	69	446

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
56	<2	7	24	0.12	90	969
58	<2	5	24	0.19	132	125
61	<2	6	24	0.14	63	NIL
62	3	9	24	0.10	50	NIL
68	2	12	59	0.25	50	10
69	<2	23	24	0.13	79	259
70	<2	5	24	0.19	69	23
72	<2	4	24	0.00	132	NIL
75	<2	7	24	0.00	61	30
77	<2	7	24	0.03	116	2252
79	2	6	24	0.00	86	487
84	<2	7	24	0.01	102	14
86	2	12	24	0.07	96	58
87	<2	8	24	0.00	49	10
88	<2	10	59	0.01	90	28
93	<2	9	32	0.14	48	16
97	<2	3	24	0.09	35	NIL
98	3	15	57	0.10	63	39
101	2	8	29	0.12	61	86
102	8	9	53	0.11	88	6
103	<2	10	50	0.11	38	47
105	<2	7	138	0.16	63	84
106	<2	8	24	0.10	196	655
107	<2	6	24	0.12	41	NIL
112	<2	8	30	0.05	46	15
114	2	8	24	0.07	41	43
117	<2	8	24	0.04	46	7
119	<2	6	24	0.00	83	1100

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
124	<2	3	31	0.01	58	40
126	2	11	75	0.09	53	12
128	<2	5	28	0.00	118	NIL
130	<2	6	35	0.00	110	111
131	<2	12	48	0.00	35	10
133	<2	6	32	0.00	40	26
137	<2	7	45	0.00	70	45
139	<2	7	28	0.00	60	14
140	<2	4	29	0.00	84	123
143	2	7	41	0.00	70	NIL
145	2	11	41	0.00	60	NIL
146	<2	6	33	0.02	51	101
151	<2	7	52	0.00	72	NIL
152	4	10	32	0.03	84	119
156	<2	13	36	0.04	65	42
158	2	15	194	0.10	47	19
160	<2	10	54	0.00	88	43
162	<2	2	26	0.00	59	45
165	<2	14	47	0.01	107	753
166	<2	12	41	0.01	107	25
170	<2	22	49	0.05	25	NIL
174	<2	8	30	0.01	97	702
175	<2	7	42	0.02	46	7
177	<2	8	26	0.01	115	67
178	<2	7	26	0.01	75	56
181	<2	12	22	0.00	83	953
182	<2	13	36	0.02	38	5
185	<2	9	27	0.02	50	NIL

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
188	<2	18	27	0.06	71	NIL
190	<2	11	29	0.01	43	43
192	<2	12	47	0.02	78	60
194	<2	11	43	0.03	96	587
196	3	10	102	0.04	92	570

Table 20. Peak serum and urine concentrations within 12 hours of onset of symptoms for patients with type 1 AMI.

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
4	50	25	144	0.05	133	4
9	90	111	1311	0.56	40	63
15	260	319	640	17.60	50	NIL
17	240	313	1611	16.30	44	62
26	70	113	356	13.40	33	81
32	400	442	718	18.20	65	185
33	180	240	2160	11.63	96	859
34	26	36	305	1.99	73	108
35	80	115	1740	2.09	44	137
36	60	92	514	1.85	30	112
39	<2	10	308	0.84	74	44
42	100	149	1608	13.10	45	78
49	27	24	104	1.10	71	42
53	240	285	3260	15.70	62	267
59	700	883	4986	21.30	67	NIL
63	39	70	361	6.80	62	54
64	42	35	678	3.99	54	15
66	130	193	409	4.80	62	14
80	110	157	1062	1.50	67	33
81	18	31	106	1.86	160	31
82	130	200	5391	3.40	36	NIL
83	46	73	537	6.90	65	NIL
89	22	41	272	0.30	65	326
92	<2	7	24	0.33	97	162
95	50	86	616	7.39	55	76
109	60	100	805	1.02	50	NIL

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
111	100	148	2490	1.04	62	13
113	9	21	138	0.25	74	15
115	42	75	1245	3.45	30	NIL
116	34	59	122	3.39	91	NIL
123	32	43	478	0.55	33	77
125	170	215	226	1.95	61	98
127	60	85	1350	4.54	85	11
135	280	397	2570	14.70	36	28
136	90	134	1860	10.60	81	NIL
138	6	45	403	1.60	40	59
141	18	32	294	1.15	39	NIL
142	110	149	1280	12.90	62	144
144	80	104	728	1.65	29	204
147	60	82	489	2.28	113	364
149	120	161	1440	4.73	73	NIL
150	160	196	2890	5.83	178	1723
153	40	66	782	0.23	49	509
154	100	142	2360	1.10	11	225
155	70	102	174	4.18	59	57
157	4	74	540	0.73	61	53
159	18	75	1370	0.33	63	20
161	320	412	4060	8.44	122	112
163	50	81	468	2.89	71	NIL
164	60	92	318	1.55	120	1045
167	150	221	2300	16.5	62	402
168	29	63	201	0.99	50	8
171	15	25	65	0.62	56	23
173	130	197	2520	1.69	60	NIL

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
176	44	72	227	0.91	91	692
179	18	42	850	0.22	76	340
187	210	265	2760	13.44	92	393
193	90	126	740	9.82	64	21

Table 21. Peak serum and urine concentrations within 12 hours of onset of symptoms for patients with type 2 AMI.

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
1	50	57	419	2.47	37	21
7	90	120	855	2.09	95	492
10	50	72	1191	1.36	50	NIL
13	19	19	113	0.11	44	23
27	33	58	226	2.71	65	185
29	11	21	60	0.30	67	174
31	70	79	322	0.53	48	256
40	80	110	658	1.51	71	34
44	10	22	26	0.79	55	29
47	8	21	24	0.45	28	36
52	80	121	105	1.70	72	51
54	20	38	282	8.94	39	37
57	13	21	78	0.37	159	88
60	18	17	212	0.17	102	337
65	41	33	137	0.48	65	242
67	8	10	55	0.32	119	36
71	16	45	352	0.70	53	30
73	220	267	4487	2.52	137	73
74	2	10	24	0.25	45	25
76	12	25	55	0.50	73	323
78	6	14	1092	0.23	228	NIL
85	80	106	1920	5.80	38	10
91	90	144	1050	5.74	75	26
94	41	79	189	0.58	73	34
96	2	15	65	0.92	72	169
99	140	240	934	9.72	49	221

Patient number	CK-MB Mass	CK-MB Activity	Myo	Troponin	Creatine (serum)	Creatine (urine)
104	26	43	287	0.66	98	36
108	<2	22	95	0.25	54	40
118	6	10	108	0.61	48	54
120	39	53	636	2.28	64	33
121	38	23	321	0.59	70	NIL
122	43	23	144	0.43	102	47
129	8	23	112	6.90	53	44
132	90	145	1360	4.20	232	562
134	22	23	107	0.45	84	53
148	25	25	340	0.94	52	46
169	36	51	112	4.10	53	NIL
172	36	94	607	0.51	45	47
180	48	47	739	2.87	56	15
183	44	86	584	0.24	32	45
184	6	14	50	0.13	49	15
186	33	48	158	1.20	118	450
189	21	49	336	1.59	75	7
191	2	14	408	0.12	62	NIL
195	35	48	138	0.38	64	12
197	8	43	343	1.42	71	22

4.1.3 ASSESSMENT OF RELATIVE DIAGNOSTIC POWER OF THE BIOCHEMICAL MARKERS

It has been established that of the biochemical markers used in this study, only CK-MB mass and activity, myoglobin and troponin-T were significantly discriminatory in the diagnosis of AMI; using peak serum concentration within 12 hours of symptom onset as the value for comparison.

In order to identify any difference in the diagnostic abilities of these markers, it was decided that diagnostic cut-offs would be established for each marker. These could then be used to determine the diagnostic accuracy of individual markers, and also allow comparison of diagnostic ability and time taken to diagnose AMI between the 4 markers.

These cut-offs were determined before the study began, and, were not altered at any stage during the study, nor modified for any of the analyses performed. They were:

CK-MB mass (Hybritech)	>5ng/ml
CK-MB activity (Kodak)	>20U/L
Myoglobin	$>100\mu$g/l
Troponin-T	>0.2ng/ml

Having established a serum concentration for each marker that was diagnostic for AMI, the biochemical data were assessed to determine which markers were able to successfully identify patients with AMI within 12 hours of the onset of symptoms, and to show which patients were incorrectly labelled as having sustained AMI. The results of this analysis are listed in tables 29-32, pages 200-209.

The success or failure of a diagnostic test are determined by its ability to discriminate between patients that do or do not have a particular pathology (Freedman,1987). The sensitivity, specificity, positive and negative predictive value are indicators of this. They are defined as follows:

$$\text{Sensitivity} = \frac{\text{True positive (and test positive)}}{\text{All true positive}}$$

i.e. the probability of a positive result in patients with AMI.

$$\text{Specificity} = \frac{\text{True negative (and test negative)}}{\text{All true negative}}$$

i.e. the probability of a negative result in patients without AMI.

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True positive and false positive}}$$

i.e. the probability that a patient has sustained an AMI if the test result is positive.

$$\text{Negative predictive value} = \frac{\text{True negative}}{\text{True negative and false negative}}$$

i.e. the probability that a patient has not sustained AMI if the test result is negative.

These measures of diagnostic accuracy were calculated for the various patient groups and are listed below:

a) DIAGNOSIS OF AMI IN ALL PATIENTS WITH AMI.

Test result (%)	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
Sensitivity	93.3	89.4	88.5	95.2
Specificity	98.8	97.7	94.3	98.9
+ve predictive value	99.0	97.9	94.8	99.0
-ve predictive value	92.4	88.5	87.2	94.5

b) DIAGNOSIS OF AMI IN GROUP A PATIENTS

Test result (%)	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
Sensitivity	91.1	94.6	92.9	98.2
Specificity	98.8	97.7	94.3	98.8
+ve predictive value	98.1	96.4	91.2	98.2
-ve predictive value	94.5	96.6	95.3	98.9

c) DIAGNOSIS OF AMI IN GROUP B PATIENTS

Test result (%)	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
Sensitivity	95.8	83.3	83.3	91.7
Specificity	98.8	97.7	94.3	98.9
+ve predictive value	97.9	95.2	88.9	97.8
-ve predictive value	97.7	91.4	91.1	95.6

d) DIAGNOSIS OF AMI IN PATIENTS WITH TYPE 1 INFARCTION

Test Result (%)	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
Sensitivity	94.8	96.6	94.8	98.3
Specificity	98.8	97.7	94.3	98.9
+ve predictive value	98.2	96.6	91.7	98.3
-ve predictive value	96.6	97.7	96.5	98.9

e) DIAGNOSIS OF AMI IN PATIENTS WITH TYPE 2 INFARCTION

Test Result (%)	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
Sensitivity	91.3	80.4	80.4	91.3
Specificity	98.8	97.7	94.3	98.8
+ve predictive value	97.7	94.9	88.1	97.7
-ve predictive value	95.6	90.4	90.1	95.6

4.1.3.1 SPECIFICITY

Overall, the specificity of the 4 biochemical markers was high, ranging from 94.3% - 98.8%. The same specificities have been listed in all 5 tables because the data for patients in group C is constant. The number of patients in group A and group B is smaller than the total number of patients sustaining AMI (as this is the sum of these 2 groups). However, as a proportion of all patients studied, the numbers of patients in these subsets are valid, and in any subsequent patient

population, are likely to be similar. Although the relatively large number of patients used to calculate specificity and, in particular, negative predictive value may be considered high, it should be noted that this relates to prediction of diagnostic accuracy for a patient in a particular subset. It is statistically more correct to use a representative sample of patients when assessing the precision of a diagnostic test (Ranshoff,1978). In the case of this study, patients being admitted to CCU with a diagnosis of presumed AMI. It could equally be argued that the specificity of any given marker with a smaller number of control subjects would be similar, because the number of false positive tests should reflect the total number of patients sampled. That is, there is no reason to suspect that the indications for admission to CCU for patients in group C should change in accordance with the AMI sub-group being studied. Those patients identified as having false-positive tests within 12 hours from the time of admission, and their probable reason for this are shown in the following table:

Table 22. PATIENTS WITH FALSE POSITIVE TESTS FOR AMI.

Biochemical Marker	Pat no	Age (y)	Sex	Result	Time after pain	Diagnosis
CK-MB Mass	102	59	M	8 ng/ml	8 hours	N.C.C.P.
CK-MB Activity	69	53	F	20.8 U/L	6 hours	U.A.
	170	73	F	21.8 U/L	2 hours	Collapse
Myoglobin	5	73	F	101 μg/l	6 hours	Collapse
	6	64	M	119 μg/l	7 hours	Collapse
	105	70	M	127 μg/l	5 hours	P.E.
	158	70	M	107 μg/l	8 hours	N.C.C.P.
	196	68	M	102 μg/l	5 hours	Collapse
Troponin-T	68	73	F	0.24 ng/ml	9 hours	U.A.

Abbreviations

Pat no = patient number
N.C.C.P. = Non-cardiac chest pain,
U.A. = Unstable angina
P.E. = Pulmonary embolism
Collapse = Collapse ?uncertain cause

4.1.3.2 COMPARISON OF THE RELATIVE DIAGNOSTIC ABILITIES OF THE FOUR BIOCHEMICAL MARKERS

The above tables indicate the actual values of sensitivity, specificity, positive and negative predictive value for the 4 markers shown to be of proven value for the diagnosis of AMI. However, these tables do not permit discrimination of one marker against another because they do not compare directly the ability to diagnose or exclude infarction for individual patients, only for each patient group as a whole. Such an analysis should be performed by a comparison of paired proportions such as McNemar's test, which compares untied observations i.e. differences rather than similarities between biochemical markers for any patient group (Daly,1991). McNemar's test was performed for all the paired group comparisons between all 4 markers. The results are shown in the following table:

Table 23. COMPARISON OF DIAGNOSTIC POWER OF 4 BIOCHEMICAL MARKERS OF AMI

Paired Markers	Patient Group	McNemar χ^2	P value
CK-MB mass CK-MB activity	All infarcts	2.7	NS
CK-MB mass Myoglobin	All infarcts	1.9	NS
CK-MB mass Troponin-T	All infarcts	0.4	NS
CK-MB activity Myoglobin	All infarcts	0.1	NS
CK-MB activity Troponin-T	All infarcts	4.5	<0.05
Myoglobin Troponin-T	All infarcts	4.6	<0.05
CK-MB mass CK-MB activity	Group A	2	NS
CK-MB mass Myoglobin	Group A	0.2	NS
CK-MB mass Troponin-T	Group A	4	<0.05
CK-MB activity Myoglobin	Group A	0.2	NS
CK-MB activity Troponin-T	Group A	2	NS
Myoglobin Troponin-T	Group A	1.8	NS
CK-MB mass CK-MB activity	Group B	6	<0.02
CK-MB mass Myoglobin	Group B	4.5	<0.05
CK-MB mass Troponin-T	Group B	0.7	NS

Paired Markers	Patient Group	McNemar χ^2	P value
CK-MB activity Myoglobin	Group B	0	NS
CK-MB activity Troponin-T	Group B	2.7	NS
Myoglobin Troponin-T	Group B	1.6	NS
CK-MB mass CK-MB activity	Type 1	1	NS
CK-MB mass Myoglobin	Type 1	0.3	NS
CK-MB mass Troponin-T	Type 1	3	NS
CK-MB activity Myoglobin	Type 1	0	NS
CK-MB activity Troponin-T	Type 1	2	NS
Myoglobin Troponin-T	Type 1	2	NS
CK-MB mass CK-MB activity	Type 2	3.6	NS
CK-MB mass Myoglobin	Type 2	4.5	<0.05
CK-MB mass Troponin-T	Type 2	0.1	NS
CK-MB activity Myoglobin	Type 2	0.1	NS
CK-MB activity Troponin-T	Type 2	2.7	NS
Myoglobin Troponin-T	Type 2	2.8	NS

4.1.3.3 DISCUSSION

The above analysis compared the accuracy of AMI diagnosis of 4 biochemical markers in comparison to a control population of patients admitted to a CCU with chest pain shown subsequently not to be AMI. The diagnosis of all patients with AMI, and smaller subgroups within this total, were considered.

For all patients with AMI, a significant difference was found between troponin-T and both CK-MB activity and myoglobin, the former being the better indicator of AMI. Troponin-T had greater values for all 4 measures of diagnostic accuracy, but especially for sensitivity, 95.2% vs 89.4% and 88.5%, and negative predictive value, 94.5% vs 88.5% and 87.2% respectively. There was a trend for troponin-T to be better than CK-MB mass, $0.05 < p < 0.10$, with troponin-T having greater values for all 4 diagnostic indicators despite statistical significance not being achieved. Overall, troponin-T is clearly the most accurate predictor of AMI within 12 hours of the onset of symptoms for all patients with AMI. This is in accordance with other researchers who advocate it as the best marker and suggest that it has superseded CK-MB as the biochemical "gold standard" of AMI diagnosis (Katus,1991;Bakker,1994).

Why troponin-T was significantly better than CK-MB activity, but not CK-MB mass is of interest. Overall, CK-MB mass was a better indicator of AMI within 12 hours of symptom onset than CK-MB activity. The 2 methods had similar specificities and positive predictive values, but CK-MB mass had greater sensitivity and negative predictive value than CK-MB activity. McNemar's test compares differences rather than similarities between pairs of markers, and it follows that overall more differences existed between CK-MB activity and troponin-T, than

between CK-MB mass and troponin-T; this was especially so for patients with type 2 infarction. This may be related to the method of measuring this enzyme. CK-MB mass detects actual quantities and gives a concentration in ng/ml. CK-MB activity measures the ability of the enzyme to catalyse a reaction, and in patients with smaller infarcts or a slower rise in enzyme concentration, this could explain why it was less good at detecting AMI within 12 hours of symptom onset.

Turning to group A, the only significant difference identified was that between troponin-T and CK-MB mass. Reference to table 29, page 200 shows that in this group, CK-MB mass was non-diagnostic for 5 patients, CK-MB activity for 3, myoglobin for 4 and troponin-T for only 1 patient. Again, because of the nature of the statistical comparison, significance was only reached for CK-MB mass. This shows the influence of such small increments of inaccurate detection of AMI. Thus, CK-MB mass failed to detect only 2 more patients than CK-MB activity, (the 2 were concordant for the 3 patients for which CK-MB activity was non-diagnostic), but against a marker with the sensitivity and specificity of troponin-T, this small difference had an effect. Why CK-MB mass should be the least accurate in this group is difficult to explain. From above it follows that it should be as least as accurate as CK-MB activity, and as patients in group A have larger infarcts overall there should not be a problem with the diagnostic threshold being reached. For 2 of the patients this however was the case, a concentration of 4ng/ml being recorded. With small numbers of patients making such a statistical difference it would be of interest to see if this phenomenon repeated itself in a similar study, or whether it was an aberration of this particular study.

The contrast in the results for group B patients is striking. The significant

differences in this subset were between CK-MB mass and CK-MB activity, and CK-MB mass and myoglobin, the former having the best diagnostic accuracy. Reference to table 30, page 203 shows that the reason for this is the considerably greater sensitivity of CK-MB mass, with it being non-diagnostic for just 2 patients compared to 8 patients for both CK-MB activity and myoglobin, and 4 patients for troponin-T. Again, it is unclear why CK-MB mass was so different in this group. Group B patients have non-diagnostic ECGs and, usually, smaller infarcts in comparison to patients in group A (Chouhan,1991). Consequently, the time taken for serum concentration to increase is greater, which explains why CK-MB activity and troponin-T with a slower rise in serum levels have relatively more patients not being diagnosed within the 12 hour time limit. However, this time factor should not apply to myoglobin, and the chosen cut-off, namely, 100μg/l is comparable to that used in earlier studies (Cairns,1983;Mair,1991a). Previous workers have shown the sensitivity of myoglobin to be lower than CK-MB for all patients with AMI. The above results suggest that this reduced diagnostic sensitivity applies specifically to a patient subgroup with smaller infarcts (who do not achieve the diagnostic cut-off within a relatively short time from the onset of symptoms), rather than to all patients with AMI, because a similar problem was not identified for patients in group A.

For patients with type 1 infarction there were no significant differences identified between any of the groups. This can be explained by the observation that for any of the 4 markers there was a maximum of 3 patients with AMI who were not correctly diagnosed. There was a trend for troponin-T to be better than CK-MB mass and myoglobin, $0.05 < p < 0.10$, but this did not achieve the required statistical significance.

It would appear that although there is a considerable overlap of patients between group A and type 1, some differences between the diagnostic abilities of the markers exist for these 2 patient subsets. The clinical significance of this is unclear, but even within as little as 12 hours from symptom onset, subtle biochemical differences appear to have developed between group A and type 1.

This observation also supports the decision to use the day 3 ECG as a reference for the type of AMI, that is QAMI or NQAMI. The above results indicate that the biochemical changes that have occurred 12 hours after symptom onset are very similar in type 1 patients, and that this seems to represent a more homogeneous group than those patients with admission ECGs diagnostic of AMI (group A). One reason for this may be that group A patients are subjected to a variety of treatments, (in particular the administration of thrombolysis), and so the opportunity to alter the pathological changes occurring in the newly ischaemic myocardium exists, presumably with effects on the serum biochemical profile. Conversely, type 1 patients probably represent a group who share a common pathological response to the effects of acute coronary occlusion, and consequently manifest a common ECG abnormality at day 3.

Patients with type 2 infarction showed similar results to those in group B. The only significant difference identified was that between CK-MB mass and myoglobin. There were trends for CK-MB mass and troponin-T to be better than CK-MB activity, $0.05 < p < 0.10$, and for troponin-T to be better than myoglobin, $0.05 < p < 0.10$. Reference to table 32, page 208 shows why this is so, with CK-MB mass failing to successfully diagnose AMI in just 4 patients, troponin-T in 5, CK-MB activity in 9 and myoglobin in 10 patients. In much the same way that a small

difference in the patients that constituted group A and type 1 had effects upon statistical significance between the various markers for these 2 groups, so too did a similar variation in those patients that constituted group B and type 2. Whereas for type 1 there were less patients incorrectly diagnosed than in group A, there were actually more patients incorrectly diagnosed in type 2 than group B. However, these increased numbers only resulted in a significant difference being identified between CK-MB mass and myoglobin, and not between CK-MB mass and activity as was noted in group B. Thus, the diagnostic abilities of the 2 assays of CK-MB were more similar for type 2 infarction than they were for patients with non-diagnostic ECG's.

From the above discussion, it follows that some patients who were not correctly diagnosed by some markers have transferred from group A to type 2. This is understandable because patients with diagnostic ECGs are more likely to receive thrombolytic therapy and so convert what initially is more likely to become a QAMI into a NQAMI. Despite this, the majority of patients with type 2 infarction are also included in group B.

In a similar way that biochemical differences were seen between patients in group A and type 1, so differences were also identified between patients in group B and type 2. Once again, this adds weight to the suggestion that pathophysiological differences develop very early from the time of coronary artery occlusion. For these 2 patient subsets in particular, these differences are likely to be related to the effects of treatment, and/or coronary artery reperfusion; be it therapeutic or spontaneous. This is because patients in group B are likely to be undertreated as the admission ECG is non-diagnostic of AMI and confirmation of infarction will be made

retrospectively by measurement of cardiac enzymes. These patients will tend to develop NQAMI, but will be joined in this group by patients with diagnostic admission ECG's who have been successfully treated with thrombolytic therapy, that is, have achieved reperfusion of the infarct related artery.

As stated previously, the numbers of patients studied were too small to show any significant differences between group A and type 1, or between group B and type 2. Further studies, biochemical or otherwise, may reveal more subtle differences within these 2 pairs of patient groups, despite their initial similar appearances.

The above discussion has compared the 4 markers with regard to their abilities to differentiate between the various groups of patients with AMI. The management of patients with acute coronary syndromes always includes the important clinical issue of excluding patients with non-cardiac chest pain. Since patients with AMI are often treated in an intensive environment, and since up to 20% of general medical admissions can be due to chest pain where a cardiac aetiology has to be confirmed or excluded, the swift, accurate diagnosis of patients with non-cardiac pathologies is of undoubted significance, especially with the cost of health care provision assuming an ever increasing importance in day to day clinical practice.

The above analysis considered peak serum concentrations within 12 hours of symptom onset. The specificities for all 4 markers were high, ranging from 94-99%, but for all patients with AMI, and for the various sub-groups, troponin-T consistently had the highest specificity and negative predictive value. Therefore, if the desired clinical endpoint was to exclude AMI, taking one blood sample 12 hours

after the onset of symptoms to measure troponin-T would achieve this with a very high degree of confidence. The analysis of troponin-T is currently the slowest of the 4 markers, although newer methods are currently being developed (Mach,1995). Allowing for this technical delay, if the most rapid analytical technique in this study was used, namely for CK-MB mass, a specificity and negative predictive value of 98.8% and 92.4% respectively could be achieved within 20 minutes of a blood sample being taken. Assuming that most patients present within a few hours of the onset of symptoms, these results show that within a few hours of admission a definite indication as to the likelihood of chest pain not having a cardiac aetiology can be made.

This analysis does not consider the question of exactly how soon after the onset of symptoms a diagnosis of acute myocardial infarction can reliably be made (or excluded); this will be considered in the next section.

4.1.4 TIME TO DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTION

4.1.4.1 COMPARISON BETWEEN MARKERS FOR SAME PATIENT GROUP

With the increased clinical need to recognise patients that have sustained myocardial infarction as soon as possible after the onset of symptoms, the data were analysed to assess any difference in the time taken to make a positive diagnosis of AMI. The earliest time that an individual marker reached the relevant diagnostic threshold was calculated. In those patients where a positive diagnosis was not made, a time of 12 hours was used. This time was decided upon because all blood samples were collected until 12 hours from symptom onset. It is of statistical importance to include all patients in the analysis and not only those that give a positive result, so as to avoid bias (Harris,1981). The data used for these calculations are listed in tables 29-32, pages 200-209. The mean times to diagnosis for the patient groups are listed below, and illustrated in the figures that follow:

Table 24. MEAN TIME + S.E.M IN HOURS AFTER THE ONSET OF SYMPTOMS TO DIAGNOSE AMI.

Patient group	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
All infarcts	5.67 ± 0.28	6.89 ± 0.32	5.32 ± 0.33	6.40 ± 0.32
Group A	5.55 ± 0.40	6.20 ± 0.38	4.82 ± 0.38	5.93 ± 0.38
Group A - T	5.57 ± 0.43	6.16 ± 0.41	4.70 ± 0.38	5.86 ± 0.41
Group A - NT	5.42 ± 0.68	6.43 ± 1.13	5.86 ± 1.42	6.43 ± 1.13
Group B	5.81 ± 0.40	7.71 ± 0.53	5.90 ± 0.57	6.96 ± 0.54
Group B - T	6.31 ± 0.68	8.00 ± 0.95	6.00 ± 1.09	7.15 ± 0.91
Group B - NT	5.63 ± 0.49	7.60 ± 0.64	5.86 ± 0.67	6.89 ± 0.67
Type 1	5.21 ± 0.36	6.12 ± 0.38	4.63 ± 0.38	5.95 ± 0.41
Type 2	6.26 ± 0.44	7.87 ± 0.52	6.17 ± 0.57	6.98 ± 0.51

Abbreviations

Group A - T = Group A patients receiving thrombolytic therapy (n=49)
Group A - NT = Group A patients not receiving thrombolytic therapy (n=7)

Group B - T = Group B patients receiving thrombolytic therapy (n=13)
Group B - NT = Group B patients not receiving thrombolytic therapy (n=35)

There were no significant differences identified between any comparisons of thrombolysed and non-thrombolysed patients for specific markers within the individual patient groups A or B. Because of the reduced numbers of patients in the thrombolysed and non-thrombolysed categories, the following analysis compared whole groups of patients. Where relevant, comments are made concerning the subgroup categories in the discussion that follows the figures below.

Figure 12. TIME TO DIAGNOSIS - ALL INFARCTS

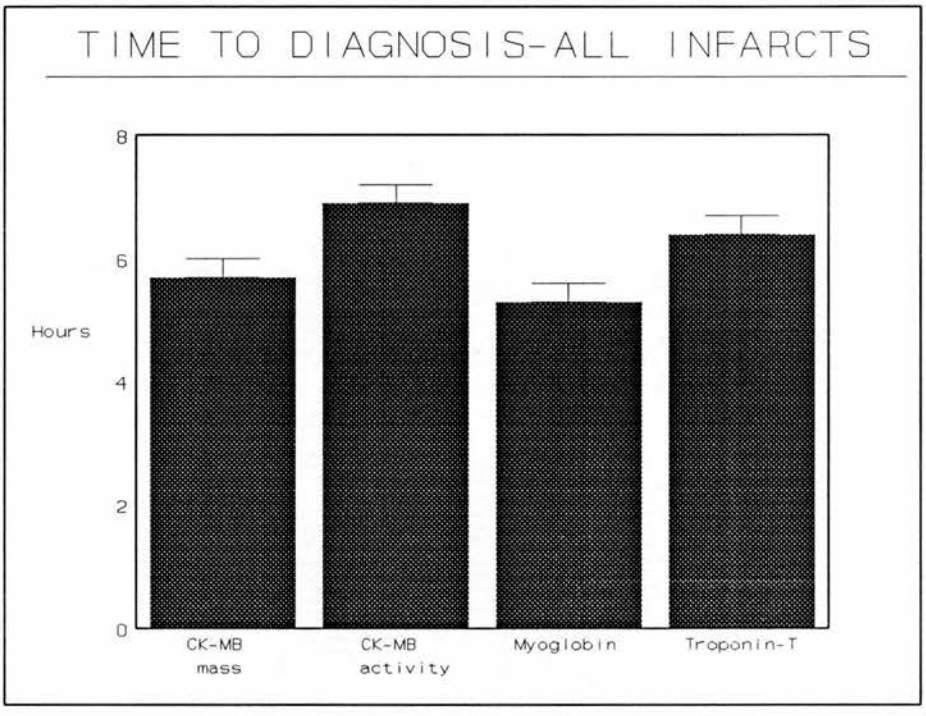


Figure 13. TIME TO DIAGNOSIS - GROUP A

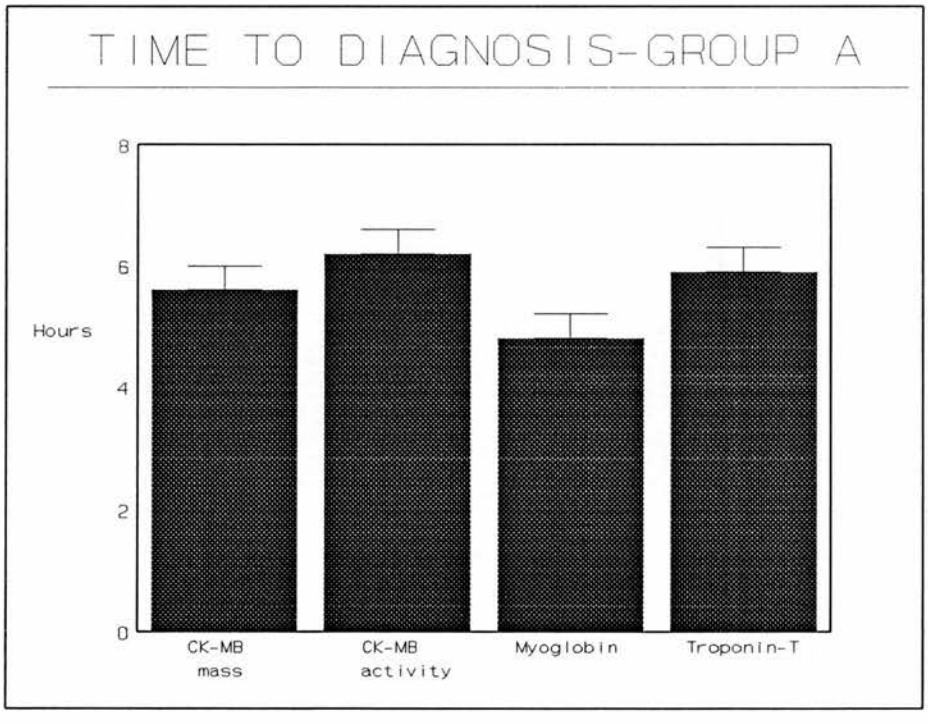


Figure 14. TIME TO DIAGNOSIS - GROUP B

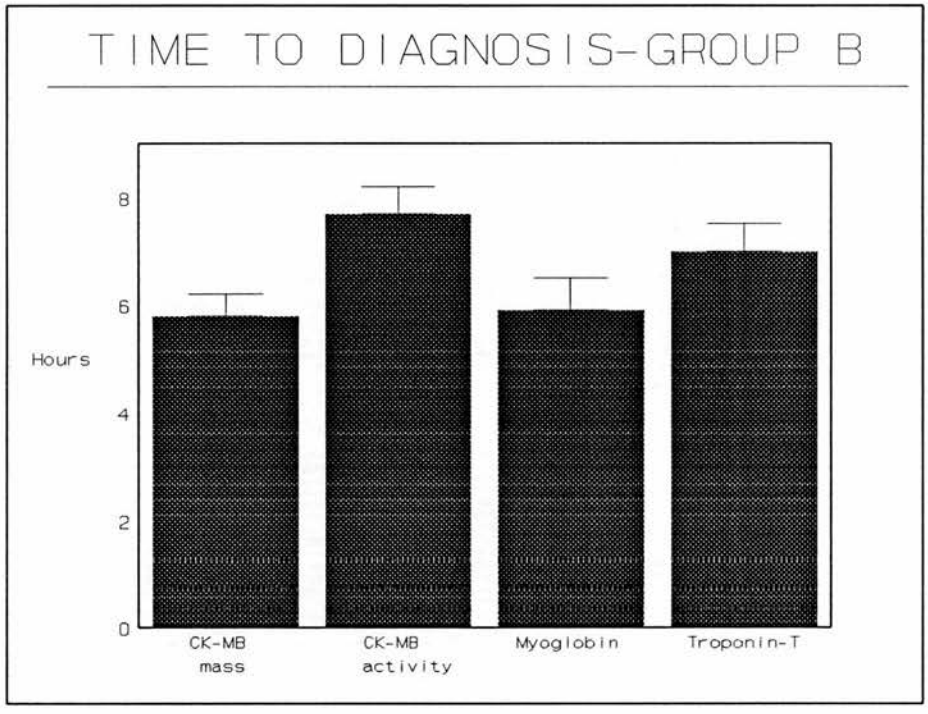


Figure 15. TIME TO DIAGNOSIS - TYPE 1 INFARCTS

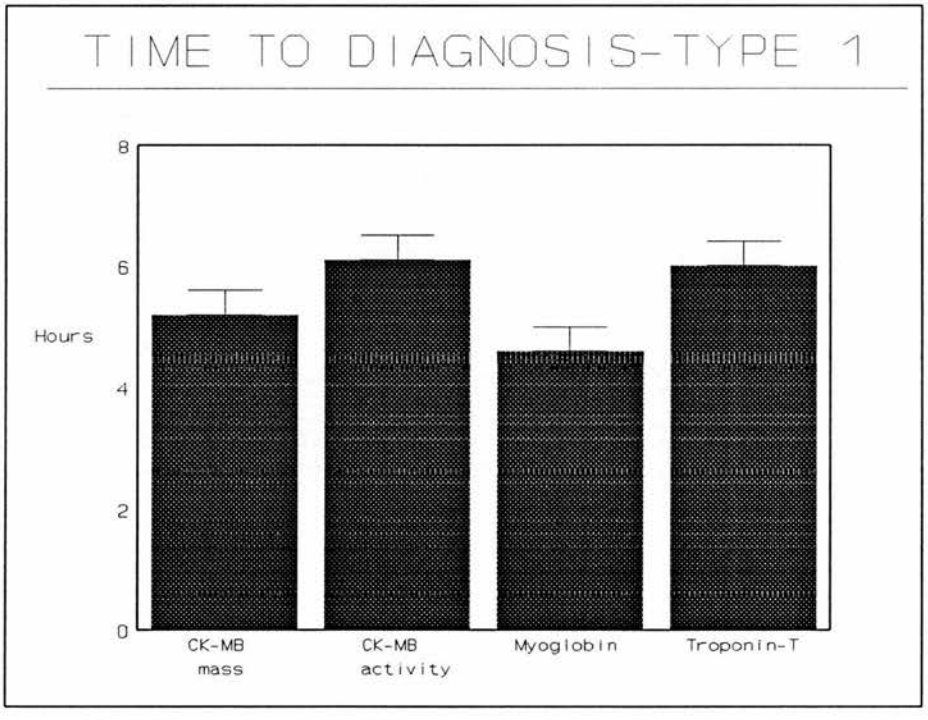
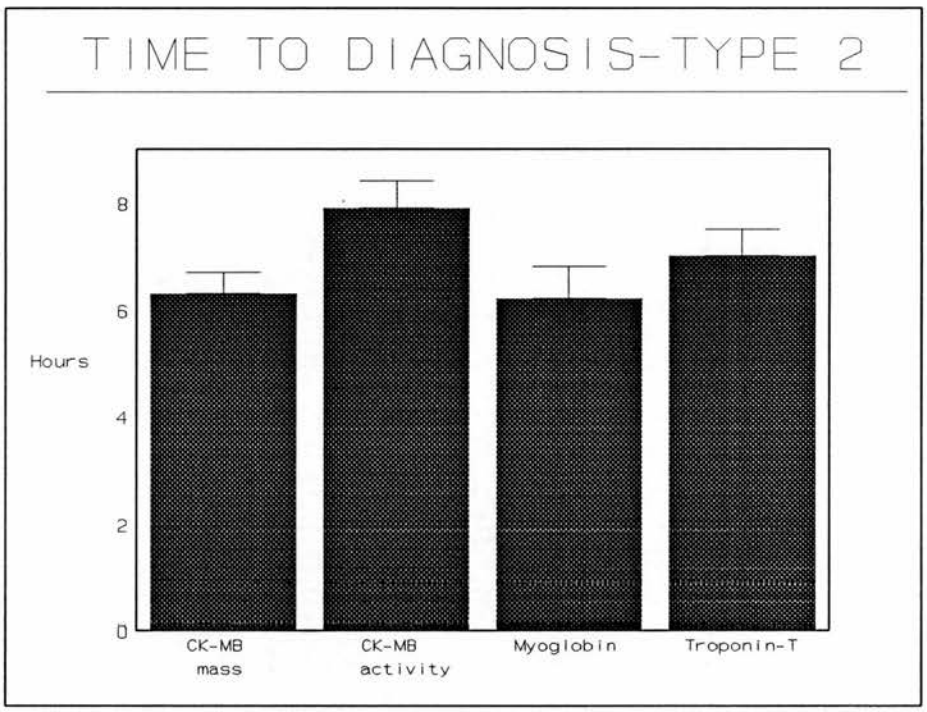


Figure 16. TIME TO DIAGNOSIS - TYPE 2 INFARCTS



The mean times to AMI diagnosis in the separate groups were assessed by ANOVA.

Table 25. ONE WAY ANOVA OF MEAN TIME TO DIAGNOSIS.

Patient group	**Degrees of freedom**	**Variance ratio**	**p value**
All infarcts	415	5.02	<0.01
Group A	223	2.71	<0.05
Group B	191	3.14	<0.05
Type 1	231	3.20	<0.05
Type 2	183	2.36	NS

Having established significant differences between the markers for the various groups, pairs of markers were compared using Student's t-test for paired samples.

Results are only given for comparisons between patient groups where a significant difference was identified.

Table 26. PAIRED MARKER COMPARISON OF TIME TO DIAGNOSIS

Paired Markers	**Patient group**	**Mean (hours)**	**Paired t statistic**	**p value**
CK-MB Mass CK-MB Activity	All infarcts	5.67 6.89	3.64	<0.001
CK-MB Activity Myoglobin	All infarcts	6.89 5.32	4.42	<0.001
Myoglobin Troponin-T	All infarcts	5.32 6.40	2.57	0.011
CK-MB Activity Myoglobin	Group A	6.20 4.82	3.15	0.003
Myoglobin Troponin-T	Group A	4.82 5.93	2.14	0.037
CK-MB Mass CK-MB Activity	Group B	5.81 7.71	3.22	0.002
CK-MB Activity Myoglobin	Group B	7.71 5.90	3.62	<0.001
CK-MB Mass CK-MB Activity	Type 1	5.21 6.12	2.14	0.036
CK-MB Activity Myoglobin	Type 1	6.12 4.63	2.88	0.006
Myoglobin Troponin-T	Type 1	4.63 5.95	2.50	0.015
CK-MB Mass CK-MB Activity	Type 2	6.26 7.87	3.03	0.004
CK-MB Activity Myoglobin	Type 2	7.87 6.17	3.16	0.003

4.1.4.1.1 DISCUSSION

For all patients with AMI (groups A and B combined), myoglobin was able to diagnose AMI more rapidly than CK-MB activity or troponin-T. Also, CK-MB mass was significantly faster than CK-MB activity. The observation that myoglobin is the most rapid indicator of AMI has been made previously (Cairns,1983), but the difference between CK-MB mass and CK-MB activity is less well known. This relates to the different methods of measurement of this enzyme. The mass method detects the presence of CK-MB in the sample, and gives a concentration accordingly. The activity method depends upon CK-MB being of sufficient concentration to catalyse a reaction. It would appear that in the first few hours after the onset of infarction, there is a lag period in which sufficient CK-MB is present to permit detection by the mass assay, but insufficient is present to reach the diagnostic cut-off chosen for the activity assay. This is not important when the time to diagnosis is irrelevant, as has been the case historically with CK-MB being the "gold standard" of AMI diagnosis (usually at day 3), but clearly, when time is important, a significant methodological difference is illustrated by this analysis.

Why myoglobin is such a rapid marker of AMI is not entirely clear. It has a molecular weight considerably lower than that of CK-MB (Kagan,1975), and hence it could be supposed that it would be released from infarcting myocytes more rapidly once the plasma membrane had become disrupted, resulting in a more rapid increase in serum concentration. As discussed above, the release kinetics of troponin-T are such that a rapid rise in serum concentration is unlikely to occur. Therefore, although it appears to be the most accurate marker for AMI diagnosis, it is of more limited value in rapid diagnosis.

Consequently, for all of the 4 markers considered to be reliable indicators of AMI, myoglobin is the best for early detection of all patients with AMI. As discussed previously, accurate exclusion of the diagnosis of AMI can be made within 12 hours of the onset of symptoms. Although myoglobin has the lowest specificity of the 4 markers evaluated in this study, it has been shown to have a specificity and negative predictive value of 94.3% and 87.2% respectively. This degree of exclusion of AMI, combined with an ability to provide this assessment within 5.5 hours of the onset of symptoms, as well as to be measured relatively rapidly in the laboratory make it suitably qualified as the best marker to provide a rapid exclusion of infarction. If any clinical doubt persists as to the validity of this, then within a couple of hours or so, troponin-T can provide an even more sensitive and specific indication as to whether or not myocardial infarction has occurred.

The time to diagnosis of AMI in patients in group A only reveals significant differences between myoglobin and troponin-T, and myoglobin and CK-MB activity. All markers had lower mean times to diagnosis for group A compared to all patients with infarction, but the relative decrease in time to diagnosis for CK-MB activity was greater than that for CK-MB mass, hence the fact that there was no significant difference between these 2, unlike for all patients with AMI. The effect of thrombolysis has to be discussed for group A patients. Analysis of the effects of reperfusion is performed in detail in chapter 5, but of the 56 patients in group A, 49 received thrombolytic therapy, compared to 13 of the 48 patients in group B. It is well known that successful reperfusion results in an earlier time to peak concentration (Blanke,1984a;Ishii,1991). Also, it has been shown that patients in group A are more likely to have QAMI, with larger infarcts. Therefore, using a

diagnostic cut-off, it is not surprising that patients in group A reach this threshold earlier than either all patients with infarction, or patients in group B. Similarly, a more rapid and larger CK-MB rise in this group will lead to sufficient enzyme being released for the diagnostic cut-off for CK-MB activity, as well as CK-MB mass, being realised, and therefore no significant difference in the time to diagnosis for these 2 markers would be expected. Similar, but not identical results to the whole of group A were seen for those patients within the group receiving thrombolytic therapy. This is not surprising since they constitute 88% of the total group. Thus, significant differences were seen between CK-MB activity and myoglobin, $p=0.001$, myoglobin and troponin-T, $p=0.023$, and in addition to the results for the whole group, myoglobin was also significantly more rapid in diagnosing AMI than CK-MB mass, $p=0.005$. However, as mentioned above, this finding is probably related to restoration of coronary artery patency and is discussed further in chapter 5.

Comparison of mean times to diagnosis for group B showed that CK-MB mass achieved diagnosis significantly earlier than CK-MB activity. Myoglobin also, was more rapid than CK-MB activity. All markers had slower times to diagnosis for group B than for all patients with AMI, and group A. This is in accordance with the above explanation, that is, patients in this group are more likely to have smaller infarcts and some will not achieve successful coronary artery reperfusion. The mean times to diagnosis were similar for myoglobin and CK-MB mass; those of CK-MB activity and troponin-T were also of similar magnitude to one another.

The release kinetics of the 2 slower markers reduce their effectiveness in the diagnosis of this important patient group, namely, those patients without diagnostic admission ECGs. Studies of thrombolytic therapy, in particular, ISIS-2, have

demonstrated that certain patients with non-diagnostic admission ECGs benefit from thrombolysis, in particular, patients with a normal ECG on admission, and those with new left bundle branch block (ISIS-2,1988). This viewpoint has been reinforced more recently (Anderson,1993). Therefore, it can be argued that early identification of these patients by biochemical means when the ECG is unhelpful is of clinical significance.

Considering the advantages of the bedside test of CK-MB mass measurement to a more complicated laboratory based system for myoglobin, there seems little doubt that until a bedside assay is developed for myoglobin with the same relative accuracy as the monoclonal antibody system used in the Hybritech ICON method, then measurement of CK-MB mass will continue to have distinct clinical advantages over myoglobin for the purpose of rapid AMI detection.

When the subgroups within group B were analysed separately, it was seen that there were no significant differences between any of the 4 markers for those patients receiving thrombolytic therapy, although as shown above, there are only 13 patients in this group. In the larger group of 35 non-thrombolysed patients, findings were similar but not identical to the group as a whole. Thus, there were significant differences seen between CK-MB activity and CK-MB mass, $p < 0.001$, and between CK-MB activity and myoglobin, $p = 0.003$. There was also a trend for CK-MB mass to achieve a more rapid diagnosis than troponin-T, $p = 0.08$. Therefore, within this group of patients with a non-diagnostic ECG on admission, in which some patients are known to benefit from the administration of thrombolytic therapy, CK-MB mass and myoglobin will achieve a diagnosis within the order of 6 hours from the time of onset of symptoms.

The results for patients with type 1 infarction are similar to those for all patients with infarction, with myoglobin being the most rapid marker, closely followed by CK-MB mass, with CK-MB activity and troponin-T having similar and significantly slower times to diagnosis. Of all the patient subgroups, the times to diagnosis are fastest for all 4 markers for type 1 infarction (note, troponin-T almost identical for type 1 and group A, 5.95 ± 0.41 and 5.93 ± 0.38 hours respectively). It is interesting to note that what is presumed to be a pathological classification of patients, namely, QAMI can be differentiated biochemically very early in its clinical course. This is even more surprising considering the fact that the ECG changes used to categorise these patients were made at day 3. This biochemical observation may have clinical validity, especially if the management of patients becomes more specific, with increasing numbers of clinical trials assessing prognosis by different management strategies in the early post-AMI period.

Patients with type 2 infarction showed similar results to those in group B. That is, myoglobin and CK-MB mass were significantly faster at diagnosing AMI than CK-MB activity. All markers without exception had the slowest times to diagnosis in this subgroup, the reasons for this have been alluded to above. These are the patients that at day 3 are shown to have NQAMI. They are constituted largely from patients in group B who have had small infarcts and/or have not achieved successful reperfusion. Although as was discussed earlier, some patients in group A were also assigned to type 2, and it was presumed that some of these had successful reperfusion, reference to table 40, page 219 shows that this was true for just 5 patients. Because of the relatively small numbers of patients in the study, and the small crossover of patients from group A to type 2 who behaved in this

way, they did not influence the overall interpretation of the times to diagnosis for patients in this sub-group. However, as for patients with QAMI, biochemical differences are seen in patients in whom a pathological difference is suspected. As has been suggested earlier, this observation has been made retrospectively. It would be of interest to test this prospectively, with pre-determined cut-offs, especially in relation to management of the various sub-groups in the early post infarct period.

4.1.4.2 ASSESSMENT OF ABILITY OF INDIVIDUAL MARKERS TO DIFFERENTIATE PATIENT SUB-GROUPS

The preceding analysis compared differences between the ability of individual markers to diagnose AMI in patient subgroups within 12 hours of the onset of symptoms. Certain differences between the release kinetics of the 4 markers were noted. However, although myoglobin may be released more rapidly than troponin-T for example, it is not clear whether there are any differences between patient groups for an individual marker. Therefore, to determine whether differences existed between the groups for any of the individual markers, an ANOVA was performed for each marker using all 4 groups. Table 24 is repeated below. Figures 17-20, which follow, show the times to diagnosis for each individual marker, and table 27 shows the results of the ANOVA.

Table 24. MEAN TIME ± S.E.M IN HOURS AFTER THE ONSET OF SYMPTOMS TO DIAGNOSE AMI.

Patient group	**CK-MB Mass**	**CK-MB Activity**	**Myoglobin**	**Troponin-T**
All infarcts	5.67 ± 0.28	6.89 ± 0.32	5.32 ± 0.33	6.40 ± 0.32
Group A	5.55 ± 0.40	6.20 ± 0.38	4.82 ± 0.38	5.93 ± 0.38
Group A - T	5.57 ± 0.43	6.16 ± 0.41	4.70 ± 0.38	5.86 ± 0.41
Group A - NT	5.42 ± 0.68	6.43 ± 1.13	5.86 ± 1.42	6.43 ± 1.13
Group B	5.81 ± 0.40	7.71 ± 0.53	5.90 ± 0.57	6.96 ± 0.54
Group B - T	6.31 ± 0.68	8.00 ± 0.95	6.00 ± 1.09	7.15 ± 0.91
Group B - NT	5.63 ± 0.49	7.60 ± 0.64	5.86 ± 0.67	6.89 ± 0.67
Type 1	5.21 ± 0.36	6.12 ± 0.38	4.63 ± 0.38	5.95 ± 0.41
Type 2	6.26 ± 0.44	7.87 ± 0.52	6.17 ± 0.57	6.98 ± 0.51

Figure 17. TIME TO DIAGNOSIS OF AMI - CK-MB MASS

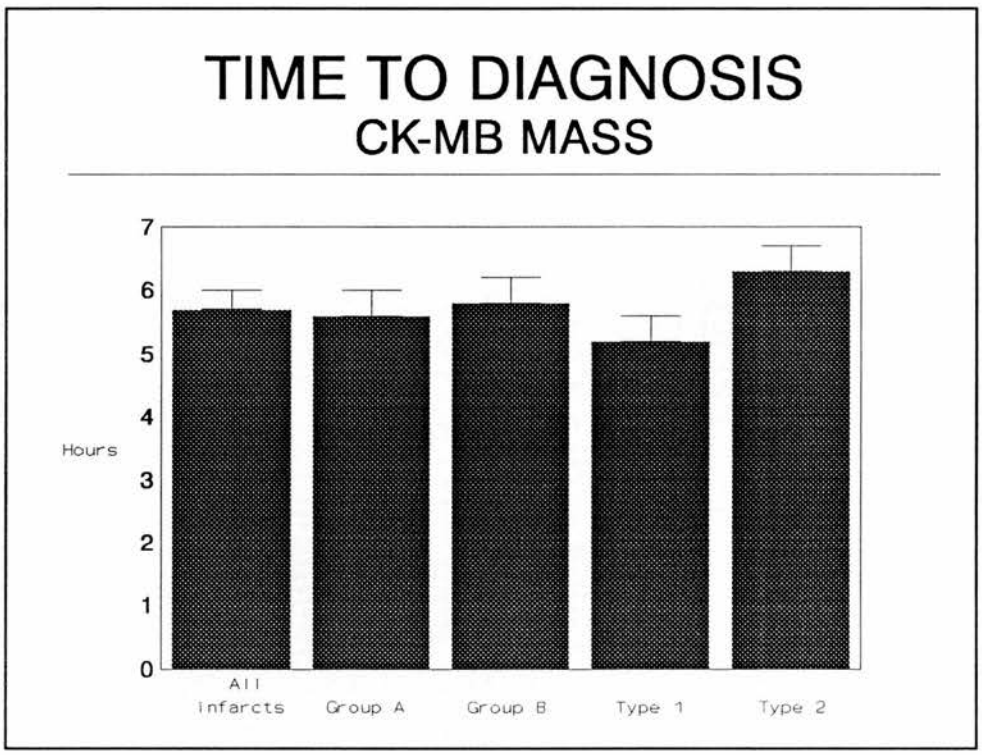


Figure 18. TIME TO DIAGNOSIS OF AMI - CK-MB ACTIVITY

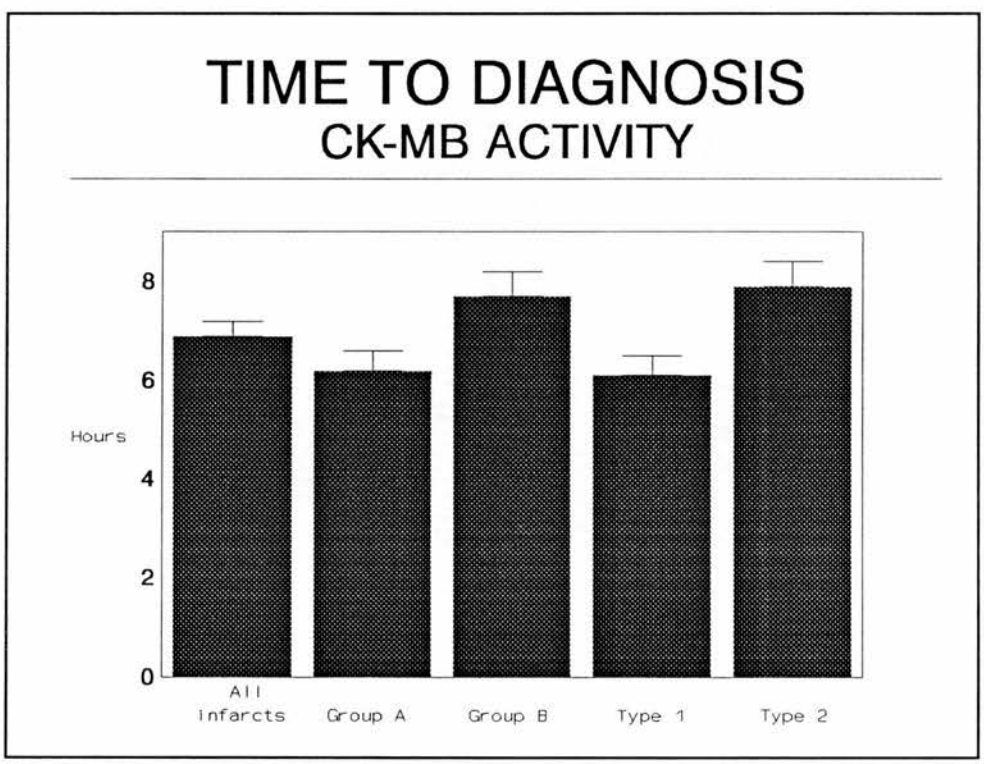


Figure 19. TIME TO DIAGNOSIS OF AMI - MYOGLOBIN

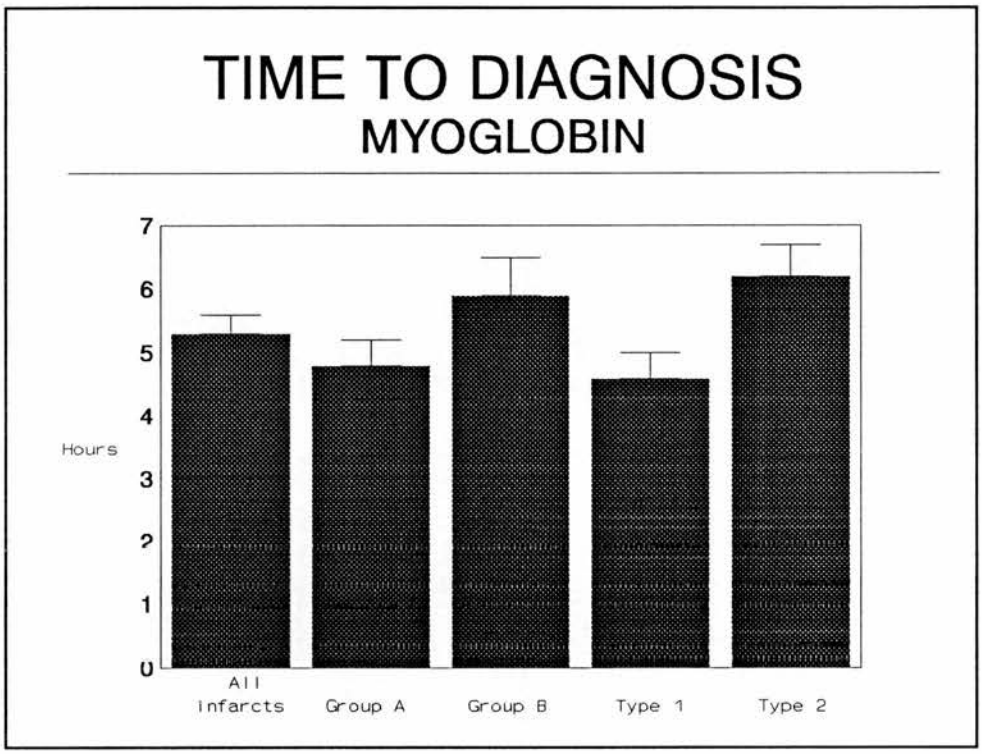


Figure 20. TIME TO DIAGNOSIS OF AMI - TROPONIN-T

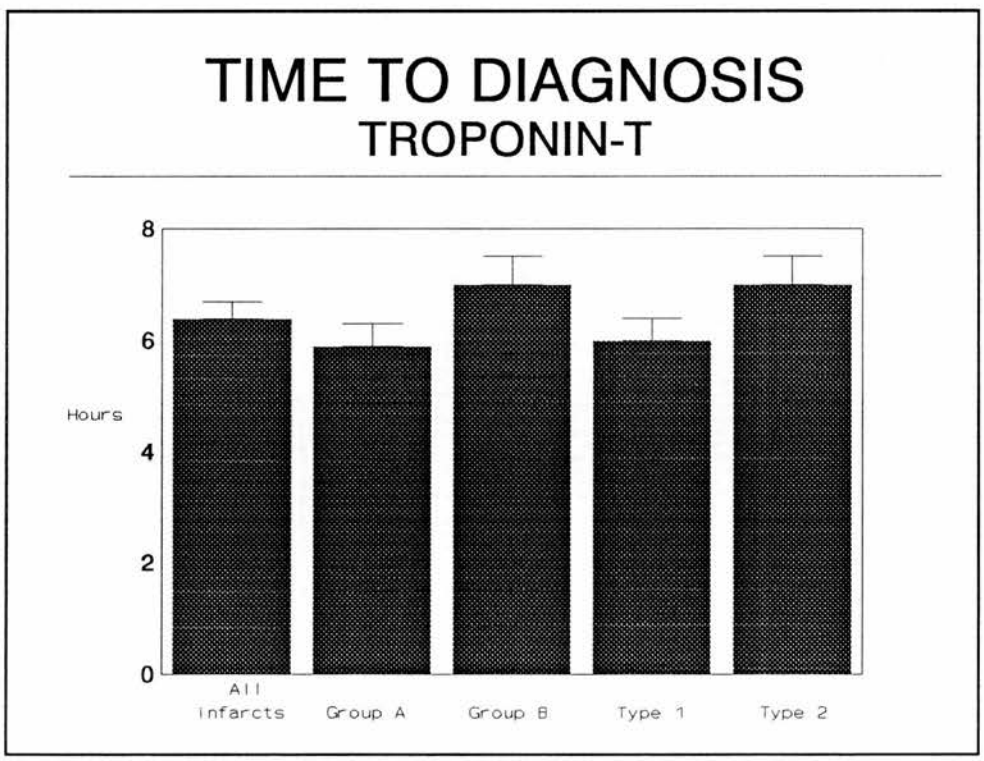


Table 27. ANOVA OF INDIVIDUAL MARKERS FOR ALL SUB-GROUPS

Biochemical marker	Degrees of freedom	Variance ratio	p value
Hybritech CK-MB	207	1.22	NS
Kodak CK-MB	207	4.41	<0.01
Myoglobin	207	2.66	<0.05
Troponin-T	207	1.69	NS

It can be appreciated that differences between the groups were only detected for CK-MB activity and myoglobin. As for previous results, having established a significant difference by ANOVA, pairs of markers were compared using Student's t-test for unpaired samples. Only positive comparisons are given in the following table:

Table 28. PAIRED COMPARISON OF TIME TO DIAGNOSIS FOR INDIVIDUAL BIOCHEMICAL MARKERS

Biochemical marker	Groups compared	Mean (hours)	t statistic	p value
CK-MB activity	Group A Group B	6.20 7.71	2.37	p=0.02
	Group A Type 2	6.20 7.90	2.64	p=0.01
	Type 1 Group B	6.12 7.71	2.49	p=0.014
	Type 1 Type 2	6.12 7.90	2.76	p=0.007
Myoglobin	Group A Type 2	4.82 6.17	2.04	p=0.04
	Type 1 Type 2	4.64 6.17	2.33	p=0.02

4.1.4.2.1 DISCUSSION

Despite the observation made in the previous discussion that the time required to diagnose AMI when compared to all patients with AMI appeared to be lowest for group A and type 1, and highest for group B and type 2, table 28 demonstrates that statistical significance for differences between the groups for an individual marker was seen for only 2 markers, CK-MB activity and myoglobin.

Since any clinical application of these observations is likely to be the differentiation of type of AMI, and since the final classification of AMI subgroup for an individual patient will depend upon the severity of infarction and subsequent response to treatment (including thrombolytic therapy), the analysis of these "within marker" differences will be considered for each patient group as a whole, rather than subdividing patients into those that did or did not receive thrombolysis or other therapies within the 3 days following their presentation. Also, the design of this study, and the numbers of patients recruited, do not provide sufficient statistical power for these various subgroup comparisons.

Firstly, considering myoglobin. Because this marker provides the most rapid overall diagnosis, it follows that it potentially has a clinical advantage if within this diagnostic window it can further differentiate between AMI subgroups.

Although a difference was seen between group A and type 2, this is of little significance, because group A is readily identified by the ECG criteria referred to earlier in this chapter (page 123). However, the observation that myoglobin can discriminate between patients with QAMI and NQAMI as early as 7 hours after the onset of symptoms is of interest. It demonstrates that the difference between these patients, who have been separated by ECG criteria at day 3, is likely to be genuine,

and further that the pathophysiological changes accompanying these 2 types of infarction are not entirely the same. It also shows that the means of deciding who has or has not had QAMI that is, all patients with Q waves (new or old) also appears to have validity.

As the management of AMI becomes more varied and selective according to patient subgroup, accurate methods for stratifying patients soon after AMI will be required. These results show that measurement of a few blood samples in the immediate post-AMI period is able to achieve this with considerable statistical significance. It is likely that other criteria will also be required to increase the accuracy of the separation into type 1 or type 2. This was decided upon at day 3, because as has been mentioned earlier, the presence or absence of Q waves in the immediate post-AMI period is not constant, and therefore the ECG is of limited use early on. These criteria are likely to consist of other biochemical indicators, a diagnostic algorithm being produced to most accurately classify patients as soon as possible after the onset of symptoms.

Also of interest are the results for CK-MB activity. This marker was the slowest of all markers used, being significantly tardier than myoglobin or CK-MB mass. However, not only was it able to discriminate between the same 2 pairs of groups as myoglobin, but it also showed a difference between groups A and B, and also type 1 and group B. The differentiation of groups A and B is of some interest because although these 2 groups of patients are readily separated by the admission ECG, the classification of patients into these 2 groups is retrospective at day 3, because criteria other than ECG changes are required to diagnose group B. The fact that patients in group B can be differentiated by biochemical criteria within 6-8

hours from the onset of symptoms, despite the ECG appearances, is of potential clinical significance.

CK-MB activity was also able to discriminate between patients with type 1 infarcts and group B. This is not surprising, considering the overlap between patients in group A and type 1, but does illustrate that patients with non-diagnostic ECGs can also be separated from patients with QAMI, and this can be achieved within a few hours of admission. It also reinforces the proposition that patients with type 1 AMI have pathological differences compared to patients in group B. The fact that patients with type 1 infarcts have an earlier time to diagnosis suggests that they are likely to have larger infarcts, with or without an increased amount of arterial reperfusion, this is usually dependent upon whether thrombolysis is administered. The effects of coronary artery reperfusion will be discussed in chapter 5.

Once again, these results demonstrate differences between the patient sub-groups of considerable statistical significance. The clinical application of these observations may be of relevance with easier and rapid bedside assay techniques being developed. The prospective validation of these findings is also required to establish appropriate diagnostic serum concentrations of these 2 markers.

4.1.5 CONCLUSIONS

1) Serum or urine creatine concentrations are not valid markers of the early diagnosis of acute myocardial infarction.

2) All 4 recognised markers, namely, CK-MB mass and activity, myoglobin and troponin-T are highly accurate in the diagnosis of all patients with AMI.

3) All 4 markers can differentiate, by serum concentration, between patients with diagnostic and non-diagnostic admission ECG's, and also between patients with Q-wave and non Q-wave myocardial infarction.

4) For all patients with AMI, troponin-T has the greatest diagnostic ability within 12 hours of the onset of symptoms.

5) For all patients with AMI, and for all 4 sub-groups myoglobin provides the most rapid diagnosis.

6) Within patient sub-groups, differentiation, by time to diagnosis, can be made by myoglobin and CK-MB activity between QAMI and NQAMI.

Table 29. TIME TO DIAGNOSIS - GROUP A PATIENTS

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
9	7	7	7	11
10	6	6	3	3
15	4	4	4	4
17	3	3	3	3
26	3	3	3	3
32	3	12	3	12
33	6	6	3	6
34	12	12	12	12
36	6	6	3	6
40	6	6	6	6
42	8	8	5	3
49	4	8	4	8
53	3	3	3	3
59	4	4	4	4
63	5	5	5	5
66	5	5	5	5
71	4	7	4	7
80	7	7	7	7
82	3	5	2	5
83	2	3	3	3
85	2	2	2	2
89	4	12	4	4
91	5	5	5	5
92	ND	ND	ND	2
96	ND	ND	ND	4
99	5	5	5	5

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
108	ND	1	ND	5
109	5	7	5	7
111	4	5	4	12
115	6	6	4	6
121	8	10	8	10
123	3	5	2	5
127	9	7	3	5
135	6	7	6	9
136	6	6	4	6
138	5	7	3	7
141	3	3	3	3
142	4	4	4	2
147	1	4	3	6
149	5	5	3	5
150	4	4	4	5
154	5	7	5	9
155	10	10	10	10
157	ND	9	5	9
159	4	5	4	8
161	2	5	2	5
163	2	2	2	2
164	6	6	6	6
167	2	6	2	4
169	8	8	8	8
171	9	9	ND	9
173	3	3	3	3
187	4	4	4	4
191	ND	ND	2	ND

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
193	3	3	2	3
197	7	9	7	9

ABBREVIATIONS

CK-MB mass = Time to diagnostic cut-off in hours from time of symptom onset

CK-MB activity= Time to diagnostic cut-off in hours from time of symptom onset

Myoglobin = Time to diagnostic cut-off in hours from time of symptom onset

Troponin-T = Time to diagnostic cut-off in hours from time of symptom onset

ND = Non-diagnostic, i.e. diagnostic cut-off not realised within 12 hours of the onset of symptoms

Table 30. TIME TO DIAGNOSIS - GROUP B AMI.

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
1	9	9	9	9
4	3	5	3	11
7	7	7	4	7
13	10	ND	10	ND
27	12	12	12	12
29	7	12	ND	ND
31	4	12	2	12
35	4	4	4	4
39	ND	ND	1	1
44	5	5	ND	5
47	9	9	ND	5
52	6	6	6	6
54	7	7	5	7
57	5	10	ND	5
60	10	ND	10	ND
64	2	2	2	2
65	4	7	4	7
67	6	ND	ND	4
73	7	11	4	11
74	ND	ND	ND	7
76	8	11	ND	5
78	3	ND	1	3
81	5	5	2	5
94	6	6	6	6
95	4	4	2	4
104	4	6	4	6

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
113	6	12	12	10
116	2	1	1	1
118	7	ND	5	2
120	3	1	1	1
122	3	12	3	9
125	9	9	9	9
129	1	1	1	1
132	1	1	1	1
134	5	5	5	12
144	6	4	4	4
148	7	9	7	12
153	8	8	8	12
168	4	6	4	6
172	6	8	3	11
176	5	7	5	7
179	8	12	6	12
180	2	4	2	4
183	5	7	4	9
184	4	ND	ND	ND
186	4	4	4	4
189	4	5	3	5
195	8	8	8	10

Table 31. TIME TO DIAGNOSIS - TYPE 1 PATIENTS

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
4	3	5	3	11
9	7	7	7	11
15	4	4	4	4
17	3	3	3	3
26	3	3	3	3
32	3	12	3	12
33	6	6	3	6
34	12	12	12	12
35	4	4	4	4
36	6	6	3	6
39	ND	ND	1	1
42	8	8	5	3
49	4	8	4	8
53	3	3	3	3
59	4	4	4	4
63	5	5	5	5
64	2	2	2	2
66	5	5	5	5
80	7	7	7	7
81	5	5	2	5
82	2	5	2	5
83	3	3	3	3
89	4	12	4	4
92	ND	ND	ND	2
95	4	4	2	4
109	5	7	5	7

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
111	4	5	4	12
113	6	12	12	10
115	6	6	4	6
116	2	1	1	1
123	3	5	2	5
125	9	9	9	9
127	9	7	3	5
135	6	7	6	9
136	6	6	4	6
138	5	7	3	7
141	3	3	3	3
142	4	4	4	2
144	6	4	4	4
147	1	4	3	6
149	5	5	3	5
150	4	4	4	5
153	8	8	8	12
154	5	7	5	9
155	10	10	10	10
157	ND	9	5	9
159	4	5	4	8
161	2	5	2	5
163	2	2	2	2
164	6	6	6	6
167	2	6	2	4
168	4	6	4	6
171	9	9	ND	9
173	3	3	3	3

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
176	5	7	5	7
179	8	12	6	12
187	4	4	4	4
193	3	3	2	3

Table 32. TIME TO DIAGNOSIS - TYPE 2 AMI.

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
1	9	9	9	9
7	7	7	4	7
10	6	6	3	3
13	10	ND	10	ND
27	12	12	12	12
29	7	12	ND	ND
31	4	12	2	12
40	6	6	6	6
44	5	5	ND	5
47	9	9	ND	5
52	6	6	6	6
54	7	7	5	7
57	5	10	ND	5
60	10	ND	10	ND
65	4	7	4	7
67	6	ND	ND	4
71	4	7	4	7
73	7	11	4	11
74	ND	ND	ND	7
76	8	11	ND	5
78	3	ND	1	3
85	2	2	2	2
91	5	5	5	5
94	6	6	6	6
96	ND	ND	2	4
99	5	5	5	5

Patient number	CK-MB Mass	CK-MB Activity	Myoglobin	Troponin-T
104	4	6	4	6
108	ND	1	ND	5
118	7	ND	5	2
120	3	1	1	1
121	8	10	8	10
122	3	12	3	9
129	1	1	1	1
132	1	1	1	1
134	5	5	5	12
148	7	9	7	12
169	8	8	8	8
172	6	8	3	11
180	2	4	2	4
183	5	7	4	9
184	4	ND	ND	ND
186	4	4	4	4
189	4	5	3	5
191	ND	ND	2	ND
195	8	8	8	10
197	7	9	7	9

CHAPTER 5: RESULTS

CORONARY ARTERY REPERFUSION

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CORONARY ARTERY REPERFUSION

5.1 ANALYSIS OF CORONARY ARTERY REPERFUSION BY ELECTROCARDIOGRAPHIC AND BIOCHEMICAL CRITERIA

5.1.1 INTRODUCTION

The need for non-invasive assessment of coronary artery reperfusion has become apparent with an ever increasing array of therapies designed to open an occluded artery. These include both medical and interventional treatments. Methods of achieving this have concentrated on 2 of the means utilised in the diagnosis of AMI, namely electrocardiographic changes, and changes in serum concentration of certain biochemical markers. In order to allow comparison between the biochemical markers used in this study it was necessary to use a technique to diagnose reperfusion that is exclusive of them (Freedman,1987). The use of coronary angiography as the "gold standard" of determining coronary artery patency at a particular moment in time is generally accepted. Although patency after thrombolysis may be variable, previous studies relating ST segment changes to early coronary angiographic appearances after thrombolysis suggest that this does allow comparison between groups of patients that have, or have not achieved successful reperfusion (Klootwijk,1993). More recently, early, complete reperfusion of the infarct related artery has been shown to be of clinically significance, immediately and 30 days after AMI (GUSTO-I,1993). Although the limitations of not using coronary angiography to document vessel patency in this study were recognised, it was still considered valid, albeit with appropriate reservations, to utilise a change in the amount of ST segment elevation after the onset of AMI as the means of determining whether or not coronary artery reperfusion had taken place.

5.1.2 CLASSIFICATION OF PATIENTS INTO SUCCESSFUL OR UNSUCCESSFUL REPERFUSION STATUS

As outlined above, in order to compare the relative properties of the 4 biochemical markers in the diagnosis of reperfusion, a means of classifying patients into those who had, or had not reperfused was required. Consideration of previous studies suggested that a sensitivity of 80-90%, and specificity of 70-80% could be realised by using appropriate ECG criteria. It was felt that if this degree of accuracy could be realised, then this would allow valid comparison of the various biochemical markers.

Therefore, since the resolution of ST segment elevation by at least 50% within 120 minutes from the administration of thrombolysis is recognised by a number of authors as being highly suggestive of recanalisation of the infarct related artery (Lee,1989; Saran,1990; Hohnloser,1991); and also incorporating information on reperfusion arrhythmias, that is disorders of cardiac rhythm thought to be related to restoring blood flow to ischaemic myocardium (Shah,1993); an analysis of all patients in group A was made.

Group A was chosen because, as defined on page 123, all patients in this group had ST segment elevation on the admission ECG. It follows from above that this is a necessary prerequisite to identify, by ECG criteria, which patients have, or have not reperfused. The classification of patients into successful or unsuccessful reperfusion is shown in table 40, page 231.

From a total of 56 patients in group A, 31 were considered to have achieved successful reperfusion. Of these 56 patients, 49 received thrombolytic therapy, and of these 49 patients, 31 successfully reperfused. None of the 7 patients not receiving

thrombolytic therapy were considered to have achieved successful reperfusion. Chi-squared analysis of the effect of thrombolysis on reperfusion status gave $\chi^2=10.05$, $p<0.005$.

The 56 patients in group A were further classified into 44 having type 1 infarction, and 12 as having type 2 infarction. Of the 44 patients with type 1, 25 had successful reperfusion; of the 12 patients with type 2, 6 achieved reperfusion. Chi-squared analysis of the association of type of infarction and reperfusion status gave $\chi^2=0.12$, p=NS.

Reviewing the ECGs for each patient in group A showed that by ECG criteria, 20 patients had an anterior infarct, and 36 an inferior infarct. Of the 20 patients with anterior MI, 11 reperfused successfully, and of the 36 patients with inferior infarction, 20 achieved reperfusion. Chi-squared analysis of the relationship between infarct location and reperfusion status gave $\chi^2=0.003$, p=NS.

5.2 EFFECT OF REPERFUSION STATUS ON BIOCHEMICAL MARKER SERUM CONCENTRATION PROFILE

It is recognised that successful reperfusion of a coronary artery results in characteristic alteration of the biochemical profile (Blanke,1984a; Ellis,1988; Remppis,1994). Biochemical data for the 4 markers in the study proven to be diagnostic for AMI, namely, CK-MB mass and activity, myoglobin and troponin-T were analysed. Serum concentrations on admission, and, 2 and 4 hours later were measured. In the 49 patients in group A in whom thrombolytic therapy was administered, serum concentrations 2 and 4 hours after thrombolysis were used for the analysis. The time to peak serum concentration (after thrombolysis or after admission) was also measured. The ratios: admission:2 hour, and admission:4 hour were calculated. These ratios, and times to peak concentration are shown in tables 41-44, pages 234-245.

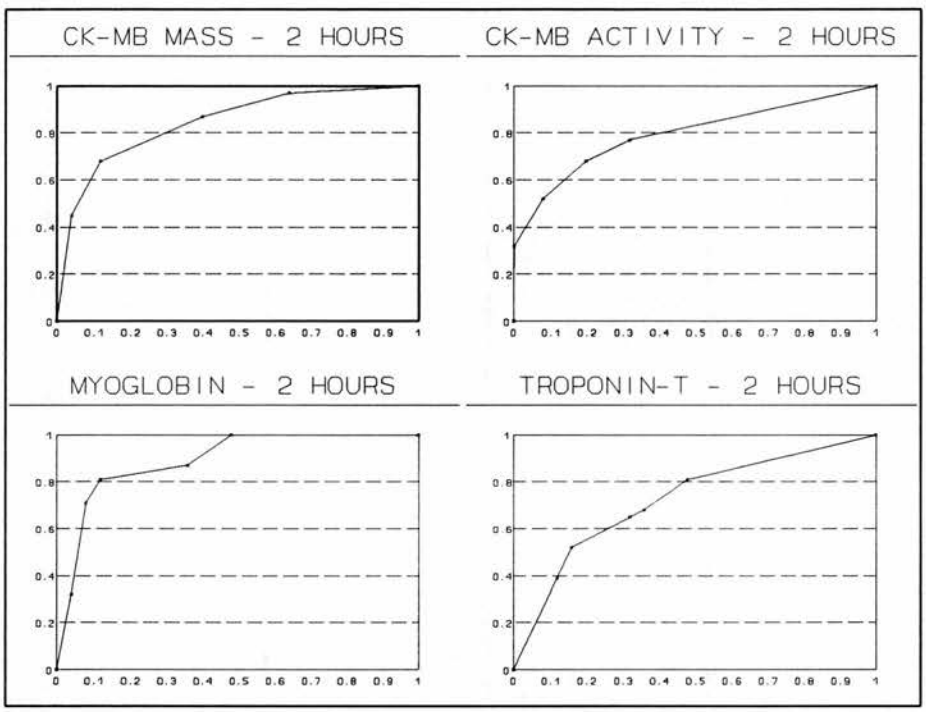
5.2.1 Determination of cut-off ratios for diagnosis of reperfusion status for each of the biochemical markers

Whereas for the diagnosis of AMI, diagnostic cut-offs had already been established prior to commencing the study, no similar diagnostic criteria were available for determining which patients had or had not achieved successful reperfusion. These were decide upon by plotting receiver operating characteristic (ROC) curves for each of the markers at 2 and 4 hours. Sensitivities and specificites were assessed for a number of ratios chosen arbritarily, these were: >1.5, >2.0, >3.0, >4.0 and >6.0. The results of this analysis are shown in the table below. These data were used to construct ROC curves for each of the 4 markers. These are shown in figures 21 and 22.

Table 33. Sensitivities and specificities of diagnosis of coronary artery reperfusion at 2 and 4 hours after the administration of thrombolytic therapy (or admission)

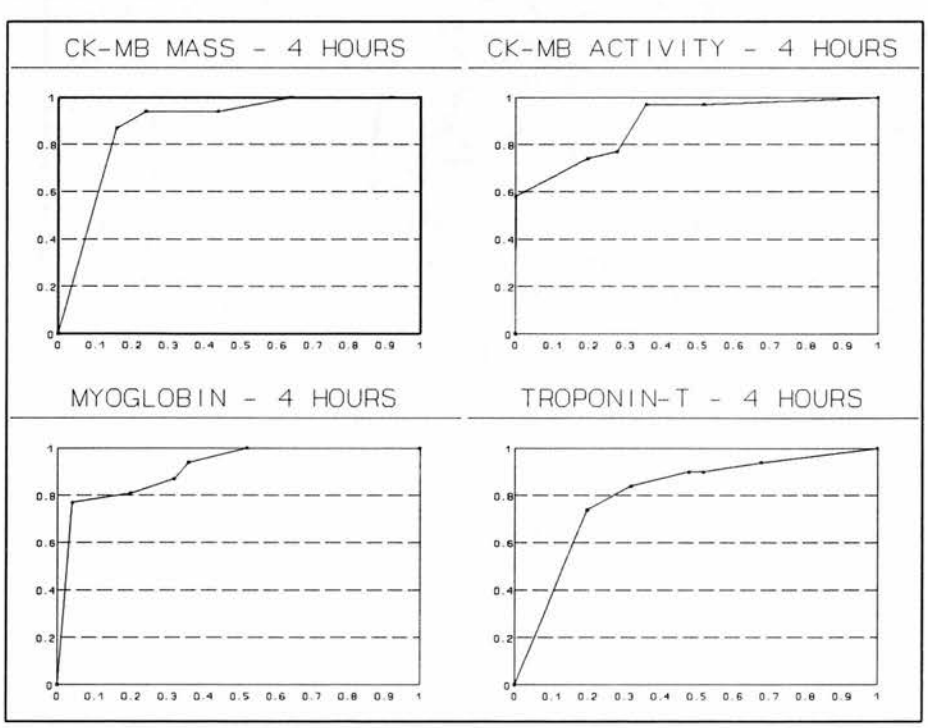
Marker (ratio)	**Sensitivity at 2 hours**	**Specificity at 2 hours**	**Sensitivity at 4 hours**	**Specificity at 4 hours**
CK-MB Mass (>1.5)	97	36	100	8
Activity (>1.5)	77	68	97	48
Myoglobin (>1.5)	100	52	100	48
Troponin (>1.5)	81	52	94	32
CK-MB mass (>2.0)	87	60	100	36
Activity (>2.0)	68	80	97	64
Myoglobin (>2.0)	87	64	94	64
Troponin (>2.0)	68	64	90	48
CK-MB mass (>3.0)	68	88	94	56
Activity (>3.0)	52	92	77	72
Myoglobin (>3.0)	81	88	87	68
Troponin (>3.0)	65	68	90	52
CK-MB mass (>4.0)	55	88	94	76
Activity (>4.0)	45	92	74	80
Myoglobin (>4.0)	71	92	81	80
Troponin (>4.0)	52	84	84	68
CK-MB mass (>6.0)	45	96	87	84
Activity (>6.0)	32	100	58	100
Myoglobin (>6.0)	58	96	77	96
Troponin (>6.0)	39	88	74	80

Figure 21. ROC CURVES FOR ALL MARKERS AT 2 HOURS



X axis = 1-specificity, Y axis = sensitivity

Figure 22. ROC CURVES FOR ALL MARKERS AT 4 HOURS



A point on each curve was chosen that best differentiated those patients that had and had not undergone coronary artery reperfusion (Hanley,1983). These are shown below. In order to maintain consistency during the subsequent analysis, once selected these diagnostic criteria were fixed and unchangeable.

Marker	**Pre:2 hour ratio**	**Pre:4 hour ratio**
CK-MB mass	>3	>4
CK-MB activity	>2	>3
Myoglobin	>3	>4
Troponin-T	>4	>4

5.2.2 COMPARISON OF INDIVIDUAL MARKER ABILITY TO DETERMINE SUCCESSFUL OR UNSUCCESSFUL REPERFUSION

A similar analysis to that used to determine any variation between the markers in the diagnosis of AMI was performed. Thus, the markers were compared to one another in order to determine any difference in their abilities to diagnose reperfusion at 2 and 4 hours after thrombolysis (or admission). The data were taken from tables 41-44, pages 234-245 and analysed by McNemar's test for comparison of paired proportions. The results are shown in the following 2 tables:

Table 34. Comparison of diagnostic accuracy of assessment of coronary artery reperfusion 2 hours after the administration of thrombolytic therapy (or admission)

Paired markers	Sensitivity	Specificity	McNemar χ^2	P value
CK-MB mass CK-MB activity	68 68	88 80	0	NS
CK-MB mass Myoglobin	68 81	88 88	1.6	NS
CK-MB mass Troponin-T	68 52	88 84	2.8	NS
CK-MB activity Myoglobin	68 81	80 88	1.3	NS
CK-MB activity Troponin-T	68 52	80 84	2.8	NS
Myoglobin Troponin-T	81 52	88 84	5.4	<0.05

Table 35. Comparison of diagnostic accuracy of the assessment of coronary artery reperfusion 4 hours after the administration of thrombolytic therapy (or admission)

Paired markers	Sensitivity	Specificity	McNemar χ^2	P value
CK-MB mass CK-MB activity	94 77	76 72	5	<0.05
CK-MB mass Myoglobin	94 81	76 80	2.7	NS
CK-MB mass Troponin-T	94 84	76 68	1.3	NS
CK-MB activity Myoglobin	77 81	72 80	0.5	NS
CK-MB activity Troponin-T	77 84	72 68	0.5	NS
Myoglobin Troponin-T	81 84	80 68	0.14	NS

5.2.3 DISCUSSION

Within 2 hours of the administration of thrombolytic therapy, one significant difference was identified between the markers with regard to identifying which patients had successfully reperfused. This was between myoglobin and troponin-T. The explanation for this was sensitivity of diagnosis, that is, 81% for myoglobin and 52% for troponin-T. The reason for the poor results for troponin-T is the slow rise in serum concentration of this marker because of its release kinetics as described earlier. Reference to table 44, page 243 shows that a large number of patients had very low ratios for troponin-T at 2 hours, because of this.

The sensitivity for both CK-MB mass and CK-MB activity was 68%, the former having a better specificity, 88% vs 80% respectively. Both of these markers had a lower sensitivity than myoglobin, but with all 3 having similar specificities, there were no statistical differences identified between them.

Both CK-MB mass and activity showed a trend towards better diagnosis than troponin-T, $0.05 < p < 0.10$. The similar findings between CK-MB mass and activity are a little surprising considering the differences seen between them with regard to the time of diagnosis of AMI. What this probably reflects is that if reperfusion takes place a large efflux of CK-MB occurs. The diagnostic threshold for CK-MB activity is then likely to be realised, and with this a sufficiently high ratio to predict successful reperfusion, although it is interesting to note that from the ROC curves, CK-MB activity had the lowest ratio for diagnosis of reperfusion of all 4 markers at both 2 and 4 hours. The similarities in diagnostic ability at 2 hours for CK-MB mass and CK-MB activity therefore would appear to be due to differences in release kinetics between where reperfusion does occur (where a large amount of CK-MB

is likely to be released quickly), compared to a slower steadier release for AMI where reperfusion does not occur. Also, choosing a fixed time interval of 2 hours does not permit the flexibility of smaller time increments used in the time to diagnose AMI analysis and so restricts any diagnostic advantage that CK-MB mass may have over CK-MB activity.

The comparison at 4 hours reveals only one significant difference, this time between CK-MB mass and CK-MB activity. The major change between the results for 2 and 4 hours is the greatly increased sensitivity for CK-MB mass. This is because a ratio has been used, and very often the initial value for CK-MB mass was less than 2ng/ml. Consequently, a relatively small rise in serum concentration is required for the ratio to increase above 4, whereas, for CK-MB activity, with a higher initial numerical concentration, allied to a slower rise in serum concentration, results in significantly poorer diagnostic accuracy; although it generally does have higher serum concentrations at 4 hours. The disadvantage to this for CK-MB mass is seen in the results for specificity, with a large number of patients having false-negative results. Consequently the specificity for CK-MB mass is lower at 4 hours compared to 2 hours, 76% vs 88% respectively. A similar phenomenon is seen for troponin-T because it too has very low initial concentrations. Although the time taken for serum concentrations to rise is greater than that for CK-MB activity (see later in this chapter), this is not reflected in the results for sensitivity which are quite high, but is seen in the results for specificity because, with so many ratios of large magnitude, there will be an increased possibility of patients with a false-negative diagnosis.

The results for myoglobin confirm what would be expected for this marker. Thus, after 4 hours, serum concentrations are at a peak and the differences in terms of ratios between reperfused and non-reperfused patients are less clear. Consequently, the sensitivity of diagnosis is the same at 4 hours as at 2 hours, that is, 81%, but the specificity has reduced from 88% to 80%. Thus, its diagnostic advantage has been removed and this is reflected in the observation that it is not significantly different to any of the other 3 markers.

As stated in the introduction to this chapter, although the limitations of using a change in the degree of ST segment elevation within 2 hours from the administration of thrombolytic therapy are recognised, within the design of this particular study, this was the main determinant of coronary artery reperfusion, which had to be decided upon independently of serum concentrations of the various biochemical markers. Separation of the 2 subgroups within group A, namely successful or unsuccessful reperfusion, was made by this non-invasive criterion, with the predicted sensitivity and specificity given above.

Therefore, the differences between the biochemical markers discussed above have to be viewed in this light. The fact that statistically significant differences were identified may be valid, but the findings in this study should be assessed with appropriate caution before they are applied in a more general manner to other patients being treated with thrombolytic therapy.

However, the fact that by using this non-invasive indicator of vessel patency all 7 of the patients in group A who did not receive thrombolytic therapy were considered not to have achieved reperfusion, and the fact that the nature of the results is in accordance with that from previous studies where angiography was

used, does suggest that there is likely to be a reasonable degree of validity of these findings. It follows that to verify them more thoroughly, they would need to be assessed prospectively and ratified by coronary angiography, performed at various times after thrombolysis.

Although coronary angiography does permit an assessment of vessel patency at any one particular moment in time, it has already been mentioned that patency is a dynamic, rather than fixed commodity. Consequently, a marker of "overall" patency, for example within the first few hours after thrombolysis may actually have more clinical value than a statement that the infarct related artery was patent at a given time, with no subsequent angiographic information as to the accuracy of that statement sometime later. Therefore, if non-invasive markers of restoration of vessel patency can be evaluated with intensive and inevitably costly studies, it may be that they will gain the upper hand ultimately in predicting which patients have achieved, and maintained, successful reperfusion status. At present this is of course hypothetical, but such an attempt to utilise ST segment variation and serum concentrations of biochemical markers, will inevitably increase dramatically the numbers of patients benefitting from the current belief of the "open artery hypothesis" with their management aimed at achieving this desired condition.

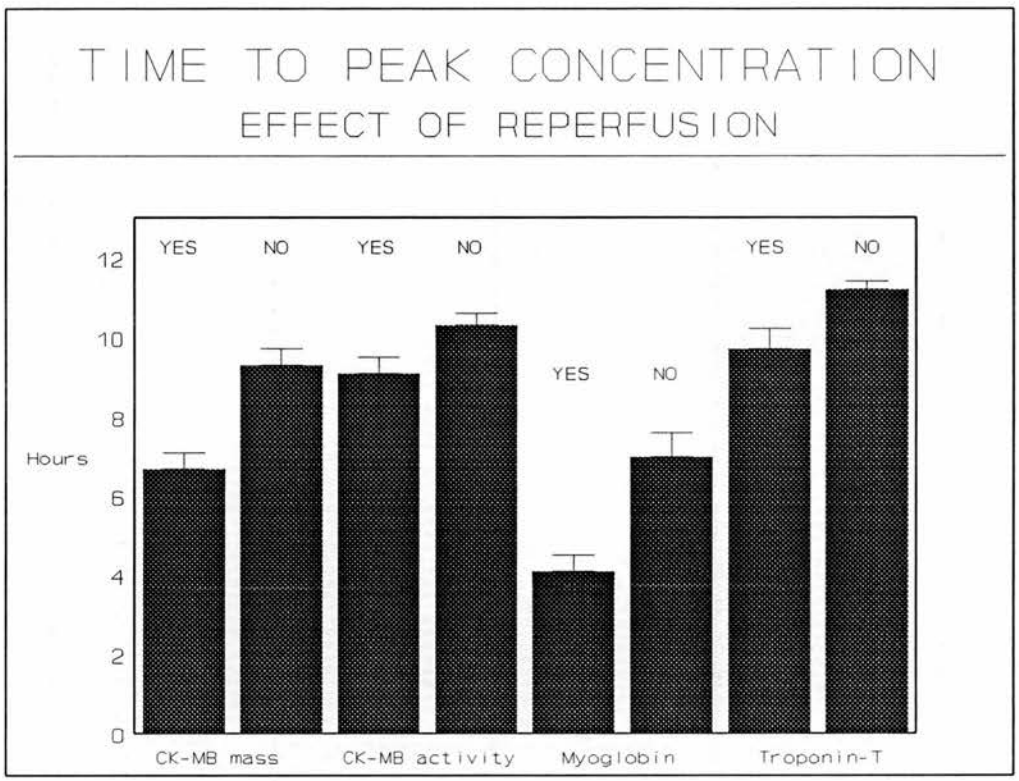
5.3 ASSOCIATION OF REPERFUSION STATUS WITH TIME TO PEAK SERUM CONCENTRATION

In addition to comparing the rate of rise in serum concentration for each marker, the time to peak concentration following thrombolysis (or admission where thrombolysis was not administered) was also assessed. The data for this analysis are listed in tables 41-44, pages 234-245. Mean concentrations of time to reach peak serum concentration are shown in table 36, and figure 23 below:

Table 36. MEAN TIME TO PEAK SERUM CONCENTRATION - GROUP A

Biochemical marker	**Reperfusion - YES Mean ± SEM (hours)**	**Reperfusion - NO Mean ± SEM (hours)**
CK-MB mass	6.71 ± 0.44	9.32 ± 0.44
CK-MB activity	9.06 ± 0.39	10.28 ± 0.30
Myoglobin	4.10 ± 0.42	7.04 ± 0.54
Troponin-T	9.68 ± 0.46	11.16 ± 0.20

Figure 23. MEAN TIME TO PEAK SERUM CONCENTRATION - GROUP A



ANOVA was performed for the mean concentrations to time to peak serum concentrations for all patients in group A, and for the 2 subdivisions within this, namely, successful and unsuccessful reperfusion status. The results are shown below:

Patient group	**Variance ratio**	**p value**
Group A - All patients	31.6	$p<0.01$
Group A - Reperfusers	35.3	$p<0.01$
Group A - Non-reperfusers	20.6	$p<0.01$

The effect of successful reperfusion on the time to peak concentration was assessed for each individual marker (table 37), and also between the 4 markers (tables 38 and 39). As previously, only significant results are listed.

Table 37. INDIVIDUAL MARKER COMPARISON OF MEAN TIME TO PEAK SERUM CONCENTRATION: REPERFUSION YES/NO

Marker	Reperfusion	No reperfusion	t statistic	p value
CK-MB mass	6.71	9.32	4.18	$p<0.001$
CK-MB activity	9.06	10.28	2.37	$p=0.021$
Myoglobin	4.10	7.04	4.37	$p<0.001$
Troponin-T	9.68	11.16	2.74	$p=0.008$

Table 38. BETWEEN MARKER COMPARISON OF MEAN TIME TO PEAK SERUM CONCENTRATION - SUCCESSFUL REPERFUSION

Paired markers	Mean time to peak (h)	t statistic	P value
CK-MB mass CK-MB activity	6.71 9.06	4.62	$p<0.001$
CK-MB mass Myoglobin	6.71 4.10	5.10	$p<0.001$
CK-MB mass Troponin-T	6.71 9.68	4.62	$p<0.001$
CK-MB activity Myoglobin	9.06 4.10	9.01	$p<0.001$
CK-MB activity Troponin-T	9.06 9.69	1.26	$p=0.217$
Myoglobin Troponin-T	4.10 9.68	10.56	$p<0.001$

Table 39. BETWEEN MARKER COMPARISON OF MEAN TIME TO PEAK SERUM CONCENTRATION - UNSUCCESSFUL REPERFUSION

Paired markers	Mean time to peak (h)	t statistic	p value
CK-MB mass CK-MB activity	9.32 10.28	2.13	$p=0.043$
CK-MB mass Myoglobin	9.32 7.04	3.99	$p<0.001$
CK-MB mass Troponin-T	9.32 11.16	3.73	$p<0.001$
CK-MB activity Myoglobin	10.28 7.04	7.29	$p<0.001$
CK-MB activity Troponin-T	10.28 11.16	3.22	$p=0.004$
Myoglobin Troponin-T	7.04 11.16	10.56	$p<0.001$

5.3.3 DISCUSSION

The results from table 37 show that for all 4 markers the time to peak serum concentration was significantly lower in those patients who achieved successful reperfusion. This is an expected finding, in accordance with previous studies, but does suggest that the electrocardiographic means used for determining coronary artery reperfusion in this study is valid, although appropriate reservation (as outlined above) should be applied to the following discussion.

A comparison between the 4 markers for patients who either did or did not reperfuse reveals similar, but not identical, findings for both sets of patients.

The time to peak serum concentration for myoglobin is significantly lower than the other 3 markers. CK-MB mass has the next lowest times, with CK-MB activity and troponin-T being significantly slower. In patients with reperfusion, CK-MB activity

and troponin-T were not significantly different, in those patients where reperfusion did not occur, CK-MB activity was significantly faster than troponin-T.

These results reinforce the findings seen in the time to diagnosis analysis, in which it was seen that serum concentration of myoglobin rises first, followed by CK-MB mass, and then CK-MB activity and troponin-T. It follows that if reperfusion occurs there will be a greater and more rapid efflux of all markers, the release kinetics of the different markers determining their relative times to peak concentration. Troponin-T overall has the slowest rise in serum concentration, this is particularly so when reperfusion does not occur.

The clinical relevance of peak serum concentrations is potentially less than that of serum concentration ratios at fixed times after the administration of thrombolysis or admission. However, with myoglobin peaking at a mean of 4.1 hours after thrombolysis, and CK-MB mass at 6.7 hours, the time window for presumed myocardial salvage is still present and can be detected easily by frequent blood sampling after thrombolysis. Once a lack of reperfusion has been demonstrated by biochemical criteria, a decision could be taken to repeat thrombolysis or proceed to alternative methods of restoring arterial patency, for example PTCA. The addition of PTCA to thrombolytic therapy for certain patient groups has been shown to be of prognostic benefit and there is increasing evidence that it should be considered where reperfusion has not occurred. The ability to identify patients who may benefit from this technique by non-invasive tests such as ECG changes or serum concentration criteria is of potential clinical benefit.

As was commented upon in the time to diagnosis discussion, the fact that CK-MB mass is measured quickly and accurately at the bedside makes this

potentially more useful clinically as it removes the delay of transfer of a serum sample to the laboratory and analysis remote from the clinical situation. If an accurate method for the measurement of myoglobin concentration at the bedside became available, the clinical advantage would be gained by this marker over CK-MB mass.

In summary, the non-invasive detection of patients who have or have not achieved successful coronary artery reperfusion can be achieved by a combination of ECG changes and characteristic patterns of serum biochemical concentrations. The fact that coronary angiography was not performed in these patients implies that the "gold standard" diagnostic technique was not performed, and once again the observations were made retrospectively. However, the prospective application of these findings to a similar group of patients, with angiographic confirmation of vessel patency, incorporating appropriate clinical action, especially in those patients in whom reperfusion is not felt to have occurred, may reveal significant effects upon prognosis for this particular patient group, the importance of which is recognised increasingly from studies such as GUSTO relating prognosis to early patency of the infarct related artery.

5.4 CONCLUSIONS

1) Two hours after the administration of thrombolytic therapy, myoglobin is significantly more accurate than troponin-T in diagnosing coronary artery reperfusion.

2) Four hours after the administration of thrombolytic therapy, CK-MB mass is significantly more accurate than CK-MB activity in diagnosing coronary artery reperfusion.

3) All 4 markers are able, by the time to peak serum concentration, to differentiate between patients who have or have not achieved successful reperfusion.

4) For those patients in whom successful reperfusion is deemed to have taken place, myoglobin has the earliest time to peak serum concentration.

Table 40. ST SEGMENT CHANGES FOLLOWING ADMISSION IN GROUP A PATIENTS

Pat no	ST elev pre	ST elev 2h post	% ST change	RHYTHM YES/NO	Reperfusion YES/NO
9	+2.48	+1.02	59	Y	Y
10	+4.32	+2.08	54	Y	Y
15	+4.96	+0.96	81	N	Y
17	+4.96	+2.40	52	Y	Y
26	-3.40	-2.60	24	N	N
32	+4.10	+1.20	71	Y	Y
33	+4.96	+2.48	50	Y	Y
34	+2.36	+1.00	58	N	Y
36	+4.76	+1.84	61	N	Y
40	+3.40	+1.20	65	Y	Y
42	+4.96	+2.16	56	Y	Y
49	+2.56	+1.80	30	N	N
53	+2.80	+1.32	53	N	Y
59	+1.60	-2.88	280	Y	Y
63	-2.04	-0.36	82	N	Y
66	+1.16	+0.84	28	N	N
71	+2.4	+1.84	23	N	N
80	+1.88	+0.96	48	N	N
82	+4.96	+1.52	69	Y	Y
83	+1.60	+0.84	48	Y	N
85	+2.16	+0.48	78	N	Y
89	+3.08	+0.28	91	N	Y
91	+1.84	+0.48	74	Y	Y
92	+1.62	+1.58	2	N	N
96	+3.5	+2.5	29	N	N
99	+1.16	+0.16	86	Y	Y

Pat no	ST elev pre	ST elev 2h post	% ST change	RHYTHM YES/NO	Reperfusion YES/NO
108	+1.52	+1.16	24	N	N
109	+3.08	-1.60	152	Y	Y
111	-4.12	-3.28	20	N	N
115	+4.96	+0.64	87	Y	Y
121	+2.80	+0.04	99	N	Y
123	+1.48	-0.06	141	N	Y
127	+1.68	+1.00	40	Y	N
135	+1.96	-0.56	129	N	Y
136	+1.68	+0.24	86	Y	Y
138	+2.52	+0.24	90	Y	Y
141	+2.1	+1.9	10	Y	N
142	+3.40	+1.20	65	Y	Y
147	+2.24	+0.70	69	N	Y
149	+1.44	+0.16	89	Y	Y
150	+4.96	+0.00	100	Y	Y
154	+1.80	+0.96	47	N	N
155	+2.20	+1.48	33	N	N
157	+2.68	+1.84	31	N	N
159	-4.40	+3.20	27	Y	N
161	+3.12	+0.64	79	N	Y
163	+1.08	+0.84	22	N	N
164	+1.24	+0.68	45	N	N
167	+4.96	+4.50	9	N	N
169	+1.80	+1.40	22	N	N
171	+2.3	+1.8	22	N	N
173	+1.56	+1.16	26	Y	N
187	+4.70	+1.20	74	Y	Y
191	+2.9	+2.7	7	Y	N

Pat no	ST elev pre	ST elev 2h post	% ST change	RHYTHM YES/NO	Reperfusion YES/NO
193	+3.10	+3.30	6	N	N
197	+1.60	+1.20	25	N	N

ABBREVIATIONS

Pat no = Patient number
ST elev pre = ST segment elevation before thrombolysis (or on admission)
ST elev 2h post admission) = ST segment elevation 2 hours after thrombolysis (or
% ST change thrombolysis = percentage ST segment change from before and after
Rhythm yes/no = presence or absence of reperfusion arrhythmia
Reperfusion yes/no reperfusion = Overall indication as to successful or unsuccessful

Table 41. CK-MB MASS CONCENTRATION RATIOS IN GROUP A PATIENTS

Pat no	Conc pre	Conc 2 h post	Conc 4 h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
9	1	2	28	2	28	12
10	4	14	32	3.5	8	6
15	1	70	180	70	180	6
17	1	70	170	70	170	6
26	38	50	70	1.3	1.8	7
32	27	150	360	5.6	13.3	6
33	1	70	110	70	110	8
34	2	4	9	2	4.5	12
36	1	1	31	1	31	6
40	1	12	60	12	60	6
42	1	3	90	3	90	7
49	8	17	27	2.1	3.3	7
53	18	80	190	4.4	10.6	7
59	1	230	440	230	440	6
63	1	29	39	29	39	4
66	30	60	100	2	3.3	8
71	7	16	30	2.3	4.3	9
80	50	80	110	1.6	2.2	11
82	5	40	70	8	14	10
83	12	30	460	2.5	3.8	9
85	14	30	80	2.1	5.7	6
89	2	5	22	2.5	11	6
91	2	23	42	11.5	21	10
92	1	1	1	1	1	12
96	<2	<2	2	2	2	10
99	1	2	40	2	40	8

Pat no	Conc pre thr	Conc 2 h post	Conc 4 h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
108	1	1	1	1	1	5
109	1	7	30	7	30	9
111	10	15	49	1.5	4.9	10
115	1	4	42	4	42	4
121	2	17	38	8.5	19	4
123	1	12	32	12	32	4
127	2	18	40	9	20	9
135	2	6	42	3	21	8
136	1	4	49	4	49	7
138	2	6	6	3	3	2
141	<2	6	14	6	14	12
142	2	24	80	12	40	5
147	18	38	50	2.1	2.8	5
149	1	4	48	4	48	10
150	8	80	160	10	20	4
154	18	40	60	2.2	3.3	11
155	28	42	58	1.5	2.1	12
157	2	4	4	2	2.	6
159	6	16	18	2.7	3	7
161	6	21	80	3.5	13.3	9
163	26	42	50	1.6	1.9	12
164	44	70	60	1.6	1.4	12
167	11	15	31	1.4	2.8	12
169	22	30	36	1.4	1.6	8
171	13	15	15	1.2	1.2	9
173	34	80	110	2.4	3.2	8
187	2	90	170	45	85	5
191	<2	2	3	2	3	12

Pat no	Conc pre thr	Conc 2 h post	Conc 4 h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
193	1	44	90	44	90	8
197	1	2	8	2	8	7

Abbreviations

Pat no = Patient number
Conc pre thr = Serum concentration before thrombolysis (or admission)
Conc 2h post = Serum concentration 2 hours after thrombolysis (or admission)
Conc 4h post = Serum concentration 4 hours after thrombolysis (or admission)
Ratio 2h:pre = Ratio of serum concentrations at 2 hours after thrombolyis (or admission), compared to serum concentration before thrombolysis (or admission)
Ratio 2h:pre = Ratio of serum concentrations at 2 hours after thrombolyis (or admission), compared to serum concentration before thrombolysis (or admission)

Table 42. CK-MB ACTIVITY RATIOS IN GROUP A PATIENTS

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
9	10	30	30	3.0	3.0	12
10	18	27	27	1.5	1.5	6
15	4	209	209	52.3	52.3	8
17	7.6	237	237	31.2	31.2	7
26	26	113	113	4.4	4.4	11
32	32	440	440	13.8	13.8	6
33	7.0	147	147	21.0	21.0	10
34	15	36	36	2.4	2.4	12
36	2.4	8.3	40	3.5	16.7	8
40	5.2	21	61	4.0	11.7	7
42	11	11.5	120	1.0	10.9	7
49	12	12	21	1.0	1.75	9
53	21	49	92	2.3	4.4	9
59	7.7	101	460	13.1	59.7	6
63	10	46	61	4.6	6.1	9
66	112	149	178	1.3	1.6	11
71	10.6	24	45	2.3	4.2	12
80	134	157	157	1.2	1.2	10
82	10	94	201	9.4	20.1	12
83	55	59	71	1.1	1.3	9
85	45	106	106	2.4	2.4	12
89	5	8	19	1.6	3.8	12
91	6	26	73	4.3	12.2	10
92	5	5	5	1.0	0.9	12
96	11.8	14.9	9.3	1.3	0.8	8
99	4.7	4	71	0.9	15.1	8

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
108	25	22	18	0.9	0.7	9
109	6	11	27	1.8	4.5	9
111	17	20	33	1.2	1.9	12
115	15	14	44	0.9	2.9	12
121	5.5	40	23	7.3	4.2	12
123	6	11	26	1.8	4.3	12
127	9.4	15	39	1.6	4.1	12
135	11	9.5	62	0.9	5.6	8
136	6.5	49	49	7.5	7.5	9
138	10.1	10.5	24	1.0	2.4	7
141	22	28	31	1.3	1.4	10
142	9.6	27	106	2.8	11.0	7
147	15	17	32	1.1	2.1	7
149	7	58	58	8.3	8.3	12
150	20	109	196	5.5	9.8	7
154	14	36	81	2.6	5.8	11
155	102	97	79	1.0	0.8	12
157	6.2	7	13	1.1	2.1	12
159	14	27	52	1.9	3.7	10
161	11	45	110	4.1	10.0	9
163	44	53	69	1.2	1.6	10
164	92	85	78	0.9	0.8	9
167	9	19	31	2.1	3.4	12
169	48	51	51	1.1	1.1	9
171	25	24	23	1.0	0.9	8
173	70	113	149	1.6	2.8	8
187	9	130	212	14.4	23.6	9
191	13	14	14	1.1	1.1	12

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
193	12	51	70	4.3	5.8	8
197	12	14	24	1.2	2.0	9

Table 43. MYOGLOBIN CONCENTRATION RATIOS IN GROUP A PATIENTS

Pat no	Conc pre	Conc 2h post	Conc 4h post thr	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
9	86.4	508	508	5.9	5.9	8
10	145	1191	1191	8.2	8.2	3
15	25	640	640	25.6	25.6	3
17	70	625	625	8.9	8.9	1
26	356	234	234	0.7	0.7	4
32	289	1040	1040	3.6	3.6	3
33	71	1719	1719	24.2	24.2	4
34	25	305	305	12.2	12.2	12
36	25	149	514	5.9	20.6	5
40	83	389	658	4.7	7.9	4
42	25	207	1608	8.3	64.3	5
49	102	104	86	1.0	0.84	5
53	401	762	906	1.9	2.3	4
59	25	4986	2163	199	86.5	2
63	25	361	327	14.4	13.1	3
66	409	328	205	0.8	0.5	4
71	352	294	197	0.8	0.6	4
80	1062	966	966	0.91	0.91	5
82	152	5391	3255	35.5	21.4	3
83	288	537	468	2.3	1.6	11
85	1188	1920	1920	1.6	1.6	2
89	27	124	94	4.6	3.5	2
91	55	1270	1050	23.1	19.1	3
92	25	25	25	1.0	1.0	12
96	212	208	174	1.0	0.8	4
99	40	74	480	1.9	12	6

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
108	49	96	100	2.0	2.1	7
109	25	269	469	10.8	18.8	7
111	127	279	570	2.2	4.5	10
115	99	375	1245	3.8	12.6	4
121	35	321	240	9.2	6.9	2
123	51	478	385	9.4	7.5	2
127	203	732	1350	3.6	6.7	8
135	25	391	1440	15.6	57.6	8
136	40	1860	1860	46.5	46.5	3
138	164	294	361	1.8	2.2	7
141	157	294	244	1.9	1.6	6
142	31	1280	672	41.3	21.7	3
147	54	172	489	3.2	9.1	3
149	82	1440	1440	17.6	17.6	3
150	2600	5500	6980	2.1	2.0	4
154	664	1190	2090	1.8	3.1	8
155	146	174	182	1.2	1.2	12
157	67	147	273	2.2	4.1	10
159	269	616	855	2.3	3.2	9
161	262	596	2010	2.3	7.7	6
163	468	347	311	0.7	0.8	5
164	318	291	231	0.9	0.7	6
167	173	737	961	4.3	5.6	10
169	112	92	92	0.8	0.8	5
171	65	65	62	1.0	1.0	6
173	1710	2520	2840	1.5	1.7	8
187	57	2330	1560	40.9	27.4	2
191	130	408	401	3.1	3.1	12

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
193	229	469	323	2.1	1.4	5
197	37	343	221	9.3	6.0	5

Table 44. TROPONIN-T CONCENTRATION RATIOS IN GROUP A PATIENTS

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
9	0.00	0.12	0.56	12.0	56	12
10	0.31	0.33	0.33	1.0	1.1	6
15	0.00	3.28	3.28	328	328	8
17	0.00	9.86	9.86	986	986	10
26	3.16	14.90	16.40	4.7	4.2	11
32	0.33	15.01	18.20	45.5	55	6
33	0.16	2.40	2.40	15.0	15.0	10
34	0.13	0.21	0.21	1.6	1.6	12
36	0.13	0.16	0.74	1.2	5.7	8
40	0.10	0.25	0.74	2.5	7.4	7
42	0.33	0.27	13.1	0.8	40	12
49	0.14	0.15	0.66	1.0	4.7	11
53	0.30	0.56	0.96	1.9	3.2	9
59	0.09	1.10	21.30	12.2	237	9
63	0.09	2.00	4.93	22.2	55	9
66	0.66	0.87	2.40	1.3	3.6	12
71	0.16	0.49	0.69	3.1	4.3	12
80	1.30	0.82	0.82	0.6	0.6	12
82	0.02	1.40	3.40	70	170	12
83	5.50	1.86	5.40	0.34	1.0	12
85	1.10	5.80	5.80	5.3	5.3	12
89	0.11	0.30	0.10	2.7	1.0	2
91	0.13	0.41	1.33	3.2	10.2	10
92	0.02	0.17	0.30	0.9	1.7	12
96	0.19	0.52	0.73	2.7	3.8	11
99	0.10	0.11	0.55	1.0	5.5	11

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
108	0.19	0.19	0.21	1.0	1.1	12
109	0.10	0.17	0.33	1.7	3.3	12
111	0.06	0.04	0.11	0.7	1.8	12
115	0.01	0.05	0.75	5.0	75	12
121	0.05	0.08	0.60	1.6	12	12
123	0.03	0.13	0.32	4.3	10.7	12
127	0.09	0.30	0.19	3.3	2.7	12
135	0.00	0.0	0.35	1.0	35	12
136	0.00	1.5	1.50	15.0	15.0	9
138	0.00	0.00	0.45	1.0	45	7
141	0.45	0.55	0.70	1.2	1.6	10
142	0.00	0.40	2.55	4.0	255	12
147	0.00	0.04	0.16	4.0	16	7
149	0.00	0.60	0.63	60	63	12
150	0.07	0.49	2.40	7.0	34	12
154	0.02	0.18	0.53	9.0	26	11
155	3.80	4.20	4.22	1.1	1.1	12
157	0.02	0.03	0.08	1.5	4.0	12
159	0.03	0.10	0.20	3.3	6.7	9
161	0.06	0.35	1.07	5.8	17.8	9
163	0.71	0.80	0.90	1.1	1.3	9
164	0.84	1.1	1.29	1.3	1.5	10
167	0.15	0.25	0.29	1.7	1.9	12
169	3.20	4.10	4.10	1.3	1.3	11
171	0.57	0.62	0.66	1.1	1.2	10
173	0.30	0.56	0.81	1.9	27	11
187	0.03	3.30	8.26	110	275	7
191	0.11	0.12	0.12	1.1	1.1	12

Pat no	Conc pre	Conc 2h post	Conc 4h post	Ratio 2h:pre	Ratio 4h:pre	Time to peak conc
193	0.03	2.60	4.20	86.7	140	10
197	0.02	0.17	0.26	8.5	13	11

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APPENDIX

PUBLICATIONS

Kelly P, Walker P, Astridge P, Ismail A, Wilson J and Perrins EJ
Troponin-T is more sensitive than creatine kinase-MB or myoglobin in diagnosing acute myocardial infarction within 12 hours from the onset of symptoms.
Eur Heart J 1993;14 Abstr Suppl:32

Kelly P, Walker P, Astridge P, Ismail A, Wilson J and Perrins EJ
Rapid measurement of creatine kinase-MB mass enables early diagnosis of acute myocardial infarction in patients with non-diagnostic electrocardiograms on admission. Eur Heart J 1993;14 Abstr Suppl:125

Kelly P, Walker P, Astridge P, Ismail A, Wilson J and Perrins EJ
Myoglobin detects acute myocardial infarction more rapidly than creatine kinase-MB or troponin-T irrespective of day 1 electrocardiogram diagnosis.
J Am Coll Cardiol 1994:78A

PRESENTATIONS

1993 European Society of Cardiology — Nice

Troponin-T is more sensitive than creatine kinase-MB or myoglobin in diagnosing acute myocardial infarction within 12 hours from the onset of symptoms.

1993 European Society of Cardiology — Nice

Rapid measurement of creatine kinase-MB mass enables early diagnosis of acute myocardial infarction in patients with non-diagnostic electrocardiograms on admission.

1994 American College of Cardiology — Atlanta

Myoglobin detects acute myocardial infarction more rapidly than creatine kinase-MB or troponin-T irrespective of day 1 electrocardiogram diagnosis.